THE UNIVERSITY
of EDINBURGH

Synaesthesia and Comorbidity

Duncan Carmichael

Doctor of Philosophy

Institute for Adaptive and Neural Computation

School of Informatics

University of Edinburgh

2015

Abstract

Synaesthesia is a hereditary, neurological condition in which common stimuli trigger unexpected secondary sensations. For example, reading letters may result in the visualisation of colour, a variant known as *grapheme-colour* synaesthesia. While synaesthesia is thought to confer a range of benefits such as improved memory, empathy, visual search and creativity to the synaesthete, there is a small, yet growing, body of evidence that suggests synaesthesia may also be associated with more clinical conditions. This thesis investigates potential associations between synaesthesia and a range of clinical conditions, identifying a set of cormorbidities, and exploring the possible genetic roots of these associations. First, I identified an increased prevalence of *multiple sclerosis (MS)* and its clinical precursor, *radiologically isolated syndrome (RIS)* in synaesthetes self-referring for participation in scientific studies. Furthermore, I identified an increased occurrence of anxiety disorder in randomly sampled synaesthetes. In addition, I show that synaesthetes with anxiety disorder experience reduced luminance in their synaesthetic colours. I also conducted an association study into the genetic origins of synaesthesia and propose the immune hypothesis of synaesthesia, which provides a theoretical basis for comorbidities (linked to the altered cortical connectivity thought to underlie the development of synaesthesia). Finally, in phenotyping synaesthesia in individuals, I also validated the most widely used online test for synaesthesia, and use this test to provide a reliable prevalence of grapheme-colour synaesthesia in the general population. Such baselines are important for establishing whether other (e.g., clinical) populations are showing rates of synaesthesia higher than otherwise expected. I also demonstrate there is no significant difference in grapheme-colour synaesthesia prevalence between the sexes and discuss its implications for genetic theories of synaesthesia.

Acknowledgements

Many people have played a hugely significant role in this PhD. Firstly, I would like to thank my supervisors Richard Shillcock and Stephen Lawrie and in particular, my primary supervisor, Julia Simner for their excellent supervision, support and good humour throughout my PhD. I couldn't have wished for better guidance and expertise, and for that I am extremely grateful.

Research conducted at the Anne Rowling clinic would not have been possible without the unfailing assistance and expertise of Belinda Weller, Anna Williams, Matt Justin and Nicola MacLeod. I would also like to express my sincere thanks to collaborators Sarah Graham and Kate Kucera for their help in explaining the mysteries of genetics. I would also like to express my gratitude to everyone who participated in my research. It goes without saying none of this would be possible without your participation.

A lot of people made academic life in Edinburgh interesting, fun and sociable. I would like to thank the staff and my fellow students at the DTC for making the past four years so enjoyable. A big thank you to Chris A. and Scotty for far-ranging whisky-fuelled chats that often went long into the night.

I would like to express my heartfelt gratitude to my family and friends for always being there, and for all the love and support you've given me over the years. Lastly, I dedicate this thesis to Zoe and Fergus. Zoe, for all your love and support, without which this thesis would never have been written and to Fergus for just being himself.

Declaration

I declare that this thesis was composed by myself, that the work contained herein is my own except where explicitly stated otherwise in the text, and that this work has not been submitted for any other degree or professional qualification except as specified.

(*Duncan Carmichael*)

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Six peer reviewed publications have arisen from this thesis

1. Simner, J. and **Carmichael, D.A.** (2015). Is synaesthesia a dominantly female trait? *Cognitive Neuroscience* (in press)

2. **Carmichael, D.A.**, Down, M.P., Shillcock, R.C., Eagleman, D.M. and Simner, J. (2015) Validating a standardised test battery for synesthesia: Does the Synesthesia Battery reliably detect synesthesia? *Consciousness and Cognition*, 33 375-385.

3. Kay, C.L., **Carmichael, D.A.**, Ruffell, H.E., and Simner J. (2014) Colour fluctuations in grapheme-colour synaesthesia: The effect of mood. *British Journal of Psychology*. doi: 10.1111/bjop.12102 .

4. *Simner, J., ***Carmichael, D.A.**, Hubbard, E.M., Morris, M. and Lawrie, S.M. (2014). Rates of white matter hyperintensities compatible with the radiological profile of multiple sclerosis within self-referred synaesthete populations. *Neurocase*, (ahead-of-print), 1-9.

5. **Carmichael, D. A.** and Simner, J. (2013). The immune hypothesis of synesthesia. *Frontiers in human neuroscience*, 7.

6. Asher, J.E. and **Carmichael, D.A.** (2013). The genetics and inheritance of synesthesia, in Simner, J. and Hubbard, E.M. (eds) *Oxford Handbook of Synesthesia*, 23-45 (Oxford: Oxford University Press).

*Joint first author

Chapter 1

Introduction

1.1 An introduction to synaesthesia

Synaesthesia is a hereditary, neurological condition in which common stimuli trigger unexpected secondary sensations. For example, listening to music may result in the visualisation of colour, in addition to the expected perception of sound, known as *music-colour* synaesthesia (Ward et al., 2006), or the visualisation of colours may be triggered by specific letters and/or numbers, a subtype known as *grapheme-colour* synaesthesia (Ward et al., 2007). In this thesis, I will ask whether having synaesthesia is also accompanied by other comorbid conditions. In this chapter, I will introduce the genetics and neurology of synaesthesia, its prevalence and sex bias and discuss how synaesthesia is empirically tested. This latter point is important for this thesis because reliable tests for synaesthesia are a prerequisite to producing accurate prevalence estimates, which is essential when comparing rates of synaesthesia in clinical and healthy populations.

The synaesthetic experience as a whole is composed of two significant components; the stimulus that triggers the secondary sensation, and the secondary sensation itself. The former is known as the *inducer*, while the latter is known as the *concurrent* (Grossenbacher and Lovelace, 2001). For example, in grapheme-colour synaesthesia,

graphemes (i.e., letters and/or numbers) are the inducer that triggers the experience of colour, which is the concurrent. In addition to the many forms of synaesthesia, there are also two distinct types of synaesthete. The distinction is based on how the concurrent sensation is experienced. Synaesthetes who experience the concurrent sensation projected outside their body are known as *projectors*, which synaesthetes who experience the concurrent internally, or in their "mind's eye", are known as *associators* (Dixon et al., 2004).

Synaesthesia is a heterogeneous phenomenon, with between 60-150 distinct variants identified to date (Cytowic and Eagleman, 2009). A broad range of inducers are found within these 60-150 variants, including such diverse stimuli as graphemes, food tastes and even pain. The resulting concurrents are similarly diverse, ranging from the experience of colour to tastes in the mouth, or the experience of emotions. While data regarding the prevalence of synaesthesia are incomplete (see section 1.2.2 below for more in-depth discussion), the most prevalent sub-type of synaesthesia is day-colour synaesthesia, experienced by an estimated 2.8% of the population, followed by grapheme-colour, with an estimated prevalence of 2% (Simner et al., 2006b)[1].

1.1.1 Neurology of synaesthesia

Synaesthesia has a neurological basis. Recent research has shown that distinct structural and functional differences exist between the synaesthetic brain and the brains of non-synaesthetes. Using a range of neuroimaging techniques, specific aspects of the neurological profile of synaesthesia have been identified, and these are reviewed in brief here.

[1]This estimate was generated by a study which excluded sequence-space synaesthesia, which may be the most prevalent type of synaesthesia, found in approximately 10% of the population (Simner, 2009; Sagiv et al., 2006) and this study also excluded mirror-touch synaesthesia, the prevalence of which is also known to be approximately equal to that of grapheme-colour synaesthesia, at 1.6% (Banissy et al., 2009a). Because the empirical work reported in this thesis was conducted primarily with grapheme-colour synaesthetes, both of these subtypes will be excluded from discussions of synaesthesia prevalence in this thesis

Structural differences affecting white matter have been discovered. These differences have been identified using *diffusion tensor imaging (DTI)*, an MRI technique which provides information about the structural integrity of white matter fibres in the brain. White matter connectivity in DTI is measured in terms of *fractional anistropy (FA)*. Simply put, FA measures the extent to which water molecules are free to diffuse in white matter tracts in the brain, on a scale of zero to one. The lower the value, the greater extent to which water molecules are free to diffuse in any direction. In healthy white matter tracts, water molecules are constrained in the direction they may diffuse by the structure of that tract. If the integrity of a tract is compromised (for example by age or disease), water molecules have greater capacity for diffusion in multiple directions, which is reflected in a lower FA value. Thus, low FA values are linked to lower integrity of white matter tracts in the brain (i.e., increases in FA indicate increases in structural white matter connectivity). Research conducted using DTI has shown localised increases in structural connectivity in the brains of synaesthetes. These increases predominately centre around the fusiform gyrus and parietal lobe (Rouw and Scholte, 2007), but have also been reported in the cingulate cortex, primary auditory cortex, frontal cortex and cerebellum (Hänggi et al., 2008). Decreases in structural connectivity have also been reported in the parietal lobe (Hänggi et al., 2008). The parietal lobe is thought to play a key role in *binding* sensory input from different modalities into a coherent whole picture.

Grey matter is also affected in synaesthesia. Evidence from structural MRI studies looking at the properties of grey matter has shown a range of structural differences in the same fusiform and intraparietal areas, including increased grey matter volume (Weiss and Fink, 2009) and increased cortical thickness and increased cortical surface area (Jäncke et al., 2009). In one study, both *increases* (in the left posterior fusiform gyrus) and *decreases* in grey matter volume (in V5 and left anterior fusiform gyrus) have been reported in the same group of synaesthetes (Banissy et al., 2012b).

As well as structural differences, functional differences have been shown to exist in the brains of synaesthetes. Evidence from functional magnetic resonance imaging (MRI) - a technique which measures cortical activity based on changes in blood flow - has shown that areas of the visual cortex that play a role in colour recognition - for example, V4 - show activation when grapheme-colour synaesthetes are presented with black-on-white graphemes (Hubbard et al., 2005; Nunn et al., 2002; Sperling et al., 2006). This suggests that, on a neuronal level, the synaesthetic brain responds to triggers to produce synaesthetically induced colour using the same mechanisms by which actual colour is perceived (Rich et al., 2006) found that when synaesthetes and non-synaesthetic controls were asked to imagine colours, both groups experienced increased neural activity in the V4 in the right hemisphere. However, when synaesthetes were presented with graphemes which induced their synaesthetic colours, this task activated the left medial lingual gyrus, activation which was not detected in non-synaesthetic controls. This suggests that different neural mechanisms may underlie the perception of colours in synaesthetes and non-synaesthetes. Further evidence that the parietal lobe plays a role in synaesthesia has been shown by the use of *transcranial magnetic stimulation (TMS)*, an experimental technique which uses electric current to temporarily stimulate localised areas of the brain. Stimulation of the right parietal lobe has been shown to disrupt synaesthetic colour-shape binding (Esterman et al., 2006) and also diminish interference in a synaesthetic Stroop test, implying that the application of TMS diminished the influence of the participants' synaesthetic colours (Muggleton et al., 2007).

The conclusions from studies conducted in a range of imaging modalities provides evidence firstly of localised structural differences in the synaesthetic cortex, manifesting itself as altered white matter connectivity and altered grey matter volume in specific locations. These regions appear to play important and specific roles in synaesthesia, such as colour processing (e.g. V4) and binding (e.g. parietal lobe) (Rouw et al., 2011).

While most research focusses on increased white matter coherence and grey matter volume in these areas, it is important to note that decreases have also been reported (Hänggi et al., 2008; Banissy et al., 2012b).These structural findings are corroborated by evidence of differing levels of functional activation found in these same areas in synaesthetes, in response to synaesthetic inducers. An interesting and open question concerns the direction of causality. It is not clear whether the structural changes to the cortex described above *cause* synaesthesia or arise as a *result of* synaesthesia (Kadosh and Walsh, 2008; Bargary and Mitchell, 2008). While a coherent picture of the neurological basis of synaesthesia appears to be emerging, it is important to point out that imaging studies in synaesthesia remains a nascent field, and not all of the findings discussed above have been replicated. For example, Jäncke et al. (2009) were not able to fully replicate the FA differences in the fusiform gyrus previously reported by Rouw and Scholte (2007). Furthermore, it has been suggested that current evidence supporting the existence of a neurological basis for synaesthesia should be interpreted with caution. Hupé and Dojat (2015) produced a comprehensive review of the MRI-synaesthesia literature and propose the view that, due to methodological limitations and low statistical power, studies in this area are yet to reliably identify any neural correlates of synaesthesia. It is important to also consider what these differences in brain structure and function may tell us about brain function in synaesthetes more broadly. It is possible that such changes may result in more widespread differences in brain function, in addition to the presence of synaesthesia. There is evidence to suggest that a range of cognitive processes differ in synaesthetes, relative to the general population. For example, synaesthetes have been shown to have improved performance in a range of memory domains, which in turn, may indicate broader differences in the way in which information is processed in the synaesthetic brain (Rothen et al., 2012). Similarly, evidence suggests that synaesthetes exhibit enhanced sensory perception more generally (Banissy et al., 2009b), which may arise as a result of general changes found in the synaesthetic brain.

1.1.2 Development and causes

This thesis looks at comorbidities in synaesthesia and therefore raises questions about its aetiology. In this section, I therefore ask what causes synaesthesia and how does synaesthesia develop? Recent research has shown synaesthesia to have a genetic basis in addition to its distinct neurological profile. Synaesthesia is an inherited condition, as first noted late in the 19th century (Galton, 1883a). Transmission from generation to generation is common, with approximately 40% of synaesthetes reporting a first-degree relative who also experiences synaesthesia (Barnett et al., 2008), although different forms of synaesthesia can occur in the same family (Ward et al., 2005). The genetic basis of synaesthesia is attracting increasing interest from researchers in the field. Currently however, research into the genetics of synaesthesia remains in its infancy, with only a handful of studies conducted to date (Asher et al., 2009; Tomson et al., 2011; Gregersen et al., 2013). These studies will be reviewed in greater detail in Chapter 4, and in Chapter 7, where I present my own genetic analysis of grapheme-colour synaesthesia. Until then, I point out that conclusions in the study of synaesthesia genetics thus far are limited - these studies have identified areas of the genome potentially linked to synaesthesia, but these areas contain several hundred possible candidate genes. To date, no genes linked to the development of synaesthesia have yet been identified.

Beyond what we actually know about genetics, the actual causes of synaesthesia remain undiscovered. There is evidence to suggest synaesthesia develops in early childhood. The *neonatal hypothesis of synaesthesia* proposes that synaesthesia is an experience common to all infants, gradually lost throughout development in the majority of people (Maurer, 1993; Spector and Maurer, 2009). According to this theory, synaesthesia is retained in synaesthetic adults due to a failure to undergo the normal pruning process. At birth, the human brain contains in the region of 100 billion neurons and many more synaptic connections than are ultimately found in the mature adult

cortex (Toga et al., 2006). During early development and into adolescence, many of these synapses are removed (i.e., "pruned") to leave a much less interconnected, but functionally more streamlined, mature brain. It is this process of synaptic removal that is proposed by the neonatal hypothesis to malfunction in synaesthetes, thus creating brains with excess connectivity. The development of synaesthesia has not yet been systematically traced through early life into adulthood. However, recent research into synaesthesia in children aged 6-11 does show that the associations between inducer and concurrent emerge slowly during childhood and strengthen over time (Simner and Bain, 2013; Simner et al., 2009a).

The majority of synaesthesia cases are thought to be developmental in nature, arising in early childhood as discussed above. However, there are other ways in which synaesthesia can occur. Temporary experiences of synaesthesia can result from taking hallucinogenic drugs such as LSD or mescaline (see Luke and Terhune, 2013 for a review). Cases of synaesthesia have also been reported as a result of neuropathological insult, such as stroke (Fornazzari et al., 2012; Ro et al., 2007). It is not known whether these acquired forms of synaesthesia are qualitatively comparable experiences to developmental synaesthesia or arise via the same neurological mechanisms (Sinke et al., 2012, but see Brogaard, 2013). There has been a suggestion that serotonin may be a common link between developmental synaesthesia and synaesthesia experienced during LSD consumption, because LSD activates a serotonin receptor (Brang and Ramachandran, 2008). In this thesis, I will only focus on developmental synaesthesia.

Two categories of model propose explanations for the creation of synaesthetic experiences (see below), and additional recent models are a hybrid of both (Brang et al., 2010). *The cross-activation model* (Ramachandran and Hubbard, 2001) suggests that activation in one cortical area (e.g., visual cortex) can trigger activation in another (e.g., auditory cortex), and this is made possible by excess connectivity between functional

areas of the cortex. The neurological evidence discussed above provides a degree of support to this model, demonstrating that altered connectivity is indeed a feature of the synaesthetic brain (e.g., Rouw and Scholte, 2007). *Re-entrant and disinhibited feedback* models propose that synaesthetic sensations are caused by disinhibited feedback from higher cortical areas (e.g., in parietal lobe) failing to suppress non-relevant activation from lower cortical areas (Grossenbacher and Lovelace, 2001). In other words, simultaneous activation arising from multiple lower sensory areas results in a synaesthetic experience. It has been suggested that this type of disinhibited feedback may result from excessive activity of excitatory neurons within the delicate balance between both excitatory and inhibitory neurons in the brain (see Hubbard et al., 2011; Terhune et al., 2011). Despite appearing superficially different, connectivity and feedback models need not be mutually exclusive. It is unlikely that altered feedback happens entirely in the absence of changes in cortical connectivity, given the Hebbian principle that simultaneous activity strengthens interconnectivity between neurons. Therefore, these two approaches might be considered somewhat unified in that connectivity models propose aberrant connectivity as the primary causal mechanism underlying synaesthesia whereas feedback models might allow altered connectivity as an indirect consequence of disinhibited feedback. While these models are now more than a decade old, explanations of how these cortical characteristics might arise have proven elusive thus far (but see Brang and Ramachandran, 2008; Mitchell, 2013).

1.2 Prevalence and objective testing of synaesthesia

In this section, I will provide a brief introduction to one area of particular interest in synaesthesia research that will be relevant to this thesis. The main topic of this thesis is comorbidities in synaesthesia. In order to address this question, several types of study were conducted; namely epidemiological research comparing the prevalence of synaesthesia in people who have certain other conditions with its prevalence in the

general population, as well as investigating the genetic make-up of verified grapheme-colour synaesthetes. Central to this approach is the requirement to objectively test for synaesthesia. Therefore the theory and practice behind this testing will itself become a matter of focus. For this reason, I will begin by discussing how testing for synaesthesia is currently conducted and I will introduce some of the problems surrounding the testing of synaesthesia. In addition, I will also describe what is known about the prevalence of synaesthesia in the general (i.e. non-morbid) population.

1.2.1 Prevalence and sex bias

One of the first questions often asked about synaesthesia is "How common is it?". A number of studies investigating this question have been conducted, with prevalence estimates that initially varied significantly. Early studies, relying exclusively on recruitment via self-declaration, estimated prevalence to be in the region of 0.05-1% of the population (Baron-Cohen et al., 1996; Barnett et al., 2008; Rich et al., 2005). By self-declaration, we mean that the experimenter places an advert asking synaesthetes to come forward, and a group of synaesthetes then self-declare in response. When participants self-declare to take part in studies, this immediately differentiates them from other members of the general public with respect to motivation to take part and their interest in the topic. As a consequence, this can have a significant influence on the outcome of scientific investigations (see below for further discussion). This problem was first identified by Simner et al. (2006b), who instead ran their prevalence study using random sampling techniques which relied less on the synaesthete's self-motivation to participate. They found the prevalence to be at least 4.4% of the population[2] (Simner et al., 2006b). This study also provided reliable estimates of the most common forms of synaesthesia, with the most prevalent sub-type of synaesthesia being day-colour synaesthesia, experienced by an estimated 2.8% of the population, followed by

[2]This study tested 128 possible variants, but excluded sequence-space synaesthesia and mirror-touch synaesthesia, now known to be common sub types (Sagiv et al., 2006; Banissy et al., 2009a)

grapheme-colour, with a estimated prevalence of 2% (Simner et al., 2006b).

Investigations into the difference in prevalence of synaesthesia between the sexes have also provided a somewhat varied picture. Initial studies reported very high numbers of female synaesthetes, (e.g. Cytowic, 1993), with ratios as high as 95% female in some cases (Baron-Cohen et al., 1993). Subsequent early studies reported significantly less skewed sex distributions, albeit with ratios that still indicate a majority of synaesthetes were female. These earlier estimates placed the sex ratio somewhere between 2:1 (Ward and Simner, 2005) and 6:1 (Baron-Cohen et al., 1996; Rich et al., 2005). This bias towards females led to early speculation that transmission of synaesthesia may be linked to the X-chromosome in some way (Baron-Cohen et al., 1996; Bailey and Johnson, 1997; Ward and Simner, 2005), but recent evidence has shown this is unlikely to be the case.

Studies showing strong female biases had again relied on self-referral, and Simner et al. (2006b) pointed out that these differences likely arose or were exaggerated by differences in self-disclosure Simner et al. (2006b). By using random sampling (rather than self-declaration), Simner et al. (2006b) found an equal prevalence of synaesthesia between men and women (1.1 females to every 1 male, a non-significant difference). Furthermore, studies investigating the genetics of synaesthesia found no evidence of linkage to the X-chromosome (Asher et al., 2009; Tomson et al., 2011). This evidence will be reviewed in greater detail in Chapter 4.

Much of the discrepancy in prevalence estimates and the ratio of synaesthesia prevalence between the sexes appears to have been introduced by the recruitment methodology and definitional criteria used in the studies concerned. Both these issues will be discussed in greater depth below.

1.2.2 Problems with synaesthesia testing

There are significant problems associated with the identification of synaesthesia in people who claim to experience it. Below, I introduce some of the issues surrounding the testing of synaesthesia that are pertinent to this thesis.

1.2.2.1 Recruitment bias

A diverse swathe of research, ranging from studies on alcohol abuse (Strohmetz et al., 1990) to participation in physical exercise (Chinn et al., 2006), supports the hypothesis that subjects volunteering to participate in scientific studies differ from the general population from which they are drawn. In general, participants are more likely to be female, well educated, intelligent and open to new experiences (a key personality trait in the "Big Five" theory of personality), in comparison to the general population (Rosenthal and Rosnow, 1975).

There is no reason to think the same bias would not affect synaesthesia research. In other words, how participants are recruited is likely to significantly influence the results of studies seeking to investigate the prevalence of synaesthesia. The less participants have the opportunity to self-select, the more accurate the prevalence estimate is likely to be. If the study relies on participants to volunteer, a range of other variables, such as individual motivation and how aware of their synaesthesia the participant is, assume greater significance.

Research into the prevalence of synaesthesia has undoubtedly been affected by these issues. In order to reach a large number of participants, early prevalence studies used print media as a recruitment tool, advertising for synaesthetes to volunteer to take part, and thus calculated prevalence based upon circulation figures of the publication in question (Baron-Cohen et al., 1996; Rich et al., 2005). For example, (Baron-Cohen et al., 1996) found 22 synaesthetes by advertising in a newspaper with a circulation of

44,000 readers, thus resulting in a prevalence estimate of 1 in 2000 (22/44,000). However, this method introduces a recruitment bias to the study, with only synaesthetes who are sufficiently self-motivated to volunteer being recorded in the results. As such, the results of these studies are undoubtedly underestimates. In contrast, the most reliable estimate of synaesthesia prevalence to date, Simner et al. (2006b), minimised this self-referral bias by first selecting a large testing cohort and then individually assessing every member of this group. With this improved method, Simner et al. (2006b) found that only approximately 1 in 80 synaesthetes in the general population will self-refer for synaesthesia studies and that these self-referrers (but not the population of synaesthetes at large) will be skewed towards females.

Simner et al. (2006b) also compared prevalence estimates from two separate populations; one consisting of university students and one recruited from visitors to a science museum. This verified that the prevalence of one form of synaesthesia (grapheme-colour) was equivalent across two samples and two testing methods (see below for a discussion of how they diagnosed grapheme-colour synaesthesia in that study).

In summary, volunteer bias is likely to have significant consequences for the prevalence of synaesthesia and the ratio of synaesthesia prevalence between the sexes. In this thesis, I will therefore test the prevalence of grapheme-colour synaesthesia, using a large sample of the population, recruited by random sampling. Volunteer bias will therefore be minimised by establishing a testing cohort and inviting prospective subjects to take part, without revealing purpose of the study beforehand (see Chapter 6).

1.2.2.2 Definitional criteria

Another reason different studies looking at synaesthesia prevalence arrived at different findings is likely to be due to how synaesthesia was defined by the study. Testing for all subtypes of synaesthesia is a logical solution to this scenario, but any study

aiming to test for all types of synaesthesia will undoubtedly be constrained to some extent by the "ease of testing" bias introduced below. Below, we shall see that some forms of synaesthesia are very difficult to objectively test and are often omitted. Furthermore, definitional criteria shift over time. For example, "sequence-personality" synaesthesia (where graphemes trigger the notion of complex personality types; e.g., A = busy mother; Simner and Holenstein, 2007), is now widely recognised as a variant of synaesthesia. However, this subtype was not included in the Simner et al. (2006b) study, because it was not widely recognised at the time. Hence, studies testing all synaesthesia subtypes can only reflect the current definition of synaesthesia at that time, thus introducing another potential source of variability to estimates of prevalence calculated at different times. Avoiding problems related to how synaesthesia is defined is a key reason that this thesis focusses on the variant of grapheme-colour synaesthesia, since this has been a clearly defined subtype for more than 100 years (see Jewanski et al., 2009).

1.2.2.3 Ease of testing bias

In this thesis, I will focus in particular on one type of synaesthesia - grapheme-colour synaesthesia - and I will briefly describe the reasons for this. As mentioned above, there are a large number of synaesthesia subtypes (e.g., Day, 2010). With regard to testing, not all subtypes have attracted equal interest. This in turn leads to certain types of synaesthesia being better understood and therefore more frequently investigated than others. The reasons are varied but are often pragmatic. Sometimes rarity is a factor - it is often not possible to recruit sufficient synaesthetes of a specific type to conduct empirical studies. This is particularly relevant to prevalence studies, which are more susceptible to the recruitment biases discussed above and later in this thesis (see Chapter 6 and Chapter 10).

Another significant reason for focussing on subtypes such as grapheme-colour

synaesthesia are ethical considerations. University researchers are duty-bound to adhere to ethical codes of conduct ensuring no harm or discomfort can arise from their experimental procedure. For this reason, synaesthesias which cause pain are seldom investigated. A further, potentially more minor, factor concerns what information the participant feels comfortable to disclose. For example, orgasm is a known synaesthetic inducer but has not yet been empirically investigated and is possibly under-reported, given its personal nature. All these reasons contribute to the heuristic that the easier a synaesthesia subtype is to test, the more it has been tested. This has significant implications when the prevalence of synaesthesia is being discussed.

Why is this important? Because of the difficulty in objectively assessing some forms of synaesthesia, it is problematic to calculate the prevalence of those subtypes. Because of this, it is difficult to estimate the overall prevalence of synaesthesia as a single phenomenon. Extrapolating the prevalence of synaesthesia as a whole by looking at a subset of types may not present a truly accurate picture. This is especially relevant given that it is currently unknown whether synaesthesia is best considered a single phenomenon or an umbrella term for a collection of behaviourally similar conditions that arise through different neurological or genetic mechanisms. I noted above that in this thesis, I focus on the specific subtype grapheme-colour synaesthesia. This subtype lends itself readily to testing, is behaviourally well understood and as a consequence, is one of the most tested and best understood forms. Thus, the prevalence measures obtained here can be readily compared to other studies in the literature.

In summary, testing for synaesthesia has certain inherent problems. Regardless of these problems, a good objective test for synaesthesia is essential. How synaesthesia is tested for is a central focus of this thesis and will be discussed in detail below.

1.2.3 Methodologies in testing for synaesthesia

Investigating the central question in this thesis - synaesthesia and comorbidity - requires reliable ways of distinguishing between synaesthetes and non-synaesthetes. As a consequence, how synaesthesia is tested for and identified becomes important to consider. The experience of a synaesthete is an inherently subjective one, personal to the individual experiencing it - the same inducers do not result in the same concurrent across all synaesthetes (Simner et al., 2005). For example, two different grapheme-colour synaesthetes may experience different colours when presented with the same letter. A significant challenge in demonstrating the genuineness of synaesthesia has been the development of meaningful and practical means of objectively evaluating this subjective experience. Such tests are of fundamental importance to synaesthesia research. Without them, we lack the tools to establish how common synaesthesia is, whether females are more affected by synaesthesia than males, and whether synaesthesia may be linked to other conditions.

Consistently describing the same concurrent as a result of perceiving the same inducer is considered to be the behavioural hallmark of synaesthesia (Baron-Cohen et al., 1987). For example, if a grapheme-colour synaesthete experiences the perception of the colour red when viewing the letter 'a', it is generally known that this association will remain consistent over years, even decades (e.g., Simner and Logie, 2008). It is this consistency that provided quantitative evidence to support the notion that synaesthesia is indeed a *bona fide* phenomenon, rather than the product of an exceptional memory or perhaps an over-active imagination. As a consequence, the majority of synaesthesia research aiming to verify the genuineness of synaesthesia relies on methods that show significant differences between the consistency of synaesthetes and non-synaesthetic controls (for example see Baron-Cohen et al., 1996; Rich et al., 2005; Simner et al., 2006b; Banissy et al., 2012b; Ward et al., 2010). Since this test of consistency as a diagnostic for synaesthesia will be at the heart of my studies in this thesis,

I briefly review its history below.

The first objective test of genuineness was designed and conducted by Baron-Cohen et al. (1987), who assessed the consistency of a single synaesthete's responses for a variety of synaesthetic inducers across a 10 week interval. The participant experienced what the authors termed "chromatic-lexical" synaesthesia, which is the term they used to describe experiencing colours induced by hearing words, names of people and individual letters (now probably termed grapheme-colour synaesthesia). The selected stimuli were 50 words, 7 days of the week, 20 Christian names and the 26 letters of the alphabet, providing a total of 103 stimuli. The accuracy of recall of the synaesthete after a 10 week interval was 100%, and this was compared to a non-synaesthetic control subject. This subject was asked to invent analogous associations and to then recall them by memory alone. The control subject achieved only 17% accuracy, with a much shorter test interval of 2 weeks. Furthermore, the retest of the synaesthete was carried out without warning, whereas the control was informed upfront about the subsequent retest.

From this starting point, an overwhelming majority of studies now verify the genuineness of their participants' synaesthesia by assessing consistency of synaesthetic response over time (e.g., Baron-Cohen et al., 1993; Baron-Cohen et al., 1996; Mattingley et al., 2001; Simner et al., 2006b) There has been, however, considerable differences in how consistency testing has been implemented. This has introduced significant variation between studies, making direct comparisons problematic. The problems associated with testing for synaesthesia using consistency are discussed in greater detail in the section below, because they will inform my methodology for verifying synaesthesia in this thesis.

The majority of studies - earlier studies in particular - employed *long term* retesting. This process involves recording a subject's synaesthetic associations at two time

points, and then comparing the consistency of response recorded on both those occasions. Time intervals of between tests can vary significantly, which is another potential source of difference between results. For example, Baron-Cohen et al. (1996) used a time interval of at least one hour, whereas Simner et al. (2006b) used a mean time interval of 6 months when testing synaesthetes (but only two weeks for control subjects, thus making the test more challenging for synaesthetes). In an effort to introduce a standardised method for assessing the genuineness of synaesthesia, Eagleman et al. (2007) produced an online battery of tests - called The Synesthesia Battery (www.synesthete.org) - that provided a way of testing consistency in a *single testing session* (I refer to this as "short-term retesting" in this thesis). This consistency test presents the relevant inducers (e.g. letters of the alphabet) three times each in a randomised order and requires the subject to pick the matching concurrent from an extensive online colour palette. The variation in colour chosen for each inducer is then quantified and averaged, providing a consistency score for each test any given subject completes. In Eagleman's test, colour is measured in RGB colour space (a colour model in which separate values for red, green and blue are combined to produce a wide range of colours, each with its own RGB vector value). The consistency score produced by the Synesthesia Battery is a measure of distance in RGB space between colours chosen by participants. Lower scores indicate smaller distance between colours in RGB space (i.e., more similar colours) and are therefore considered more consistent and thus more synaesthetic. In this way, the final score can be considered a "distance" score, with a low score meaning a small distance (in colour space) between the three selections of colour for each grapheme (where small distance means highly consistent responding). There are tests for a range of synaesthetic inducers including graphemes, days of the week, months and musical notes and chords but the test is limited to subtypes of synaesthesia that have colour as the concurrent. One recent study has built upon Eagleman's work and speculated that the threshold in the Synesthesia Battery perhaps should be raised. Rothen et al. (2013) have recently argued that a

more appropriate cut-off may indeed be higher, and their argument relates to considerations of colour space. Eagleman et al. (2007) evaluates consistency using distance in RGB space but Rothen et al. (2013) suggest that alternative colour models (CIELUV and CIELAB) might provide more sensitive measures. On this basis they proposed a revised threshold within the existing Synesthesia Battery at < 1.43 for synaesthetes (rather than < 1).

There are significant advantages to this short-term consistency approach. Firstly, a quantitative consistency score is calculated, providing an objective, easily comparable measure of consistency. Testing can be completed in a single session, resulting in increased practicality and reducing the risk of participant drop out. The fact the test is hosted entirely online allows participants to be tested remotely, greatly increasing the scope for recruiting larger samples of subjects and potentially increasing the opportunities for collaboration between researchers. In this thesis, I will use this consistency methodology when assessing synaesthesia (both for epidemiological studies and for phenotyping participants in a genetics study) and will tend to use the short term approach unless particular constraints require a long-term approach (see Chapter 8). Furthermore, since the short-term consistency approach has never been evaluated in comparison to the more traditional long-term consistency approach, I also dedicate one of my empirical chapters to this aim. For a more in-depth explanation of this test and my evaluation of it, please see Chapter 6.

1.2.4 Thesis overview

To conclude this chapter, I will summarise the contents of this thesis. The aim of this thesis is to explore whether synaesthesia has any comorbidities. That is to say, whether it co-occurs with any other conditions at greater levels than would be found in the general population. I begin Chapter 2 by reviewing and summarising - for the first time - all the existing literature on conditions that may (or may not) co-occur with

synaesthesia.

In Chapter 3, I focus on one particular condition - multiple sclerosis - and present data demonstrating that multiple sclerosis and its clinical precursor, *radiologically isolated syndrome* appear to be more prevalent in synaesthetes self-referring to participate in scientific studies than in the general population.

In Chapter 4, I consider the same question from a genetics viewpoint. I first provide a general introduction to genetics, a detailed review of the synaesthesia genetics literature, and the genetics of multiple sclerosis, and I explore why the two conditions may share common genetic origins.

In Chapter 5 I present my *immune hypothesis of synaesthesia*, as a theoretical model to predict which conditions may co-occur in synaesthesia. This model explores a theoretical framework explaining how the origins of synaesthesia may lie with genes that play an important role in the development of the cortex in the first years of life, and which have an immune function in the adult brain.

In Chapters 6 and 7 I present my own investigation into the genetics of synaesthesia which has the single aim of better understanding comorbidity by examining the roots of synaesthesia, and several related threads. These involve first establishing (in Chapter 6), a valid way to phenotype synaesthesia by evaluating an existing online testing platform (The Synesthesia Battery; Eagleman et al., 2007). This validation involves using the platform to generate the most accurate and large scale assessment of the prevalence of grapheme-colour synaesthesia to date, and then comparing this to a previous most widely accepted prevalence figure established from a well-validated study using a different method (Simner et al., 2006b). This approach will also provide the most accurate estimate of sex ratios within synaesthesia, which will itself be used in my discussions of comorbidity (i.e., is synaesthesia more common in women? Is a sex bias found in any other potential comorbidities?). In Chapter 7, I will use this

(now validated) method of testing for synaesthesia to phenotype participants in my own investigation into the genetics of synaesthesia.

In Chapter 8, I present data exploring the prevalence of grapheme-colour synaesthesia in a sample of patients with multiple sclerosis and in Chapter 9, I address a different area of comorbidity by presenting empirical work showing that anxiety disorder is more prevalent in randomly sampled synaesthetes when compared to non-synaesthetes. In Chapter 10, I discuss the conclusions reached by this thesis, and explore future directions of research in this area.

Chapter 2

A review of synaesthesia and comorbidity

2.1 Introduction

Synaesthesia is generally considered to be a benign alternative form of perception, more often thought to confer cognitive benefits to the synaesthete, rather than negative outcomes. A broad range of advantages have been suggested, including benefits in memory (Rothen et al., 2012), empathy (Banissy and Ward, 2007), visual search and creativity (Ramachandran and Hubbard, 2001). The traditional portrayal of synaesthesia in the scientific literature reflects this view. At time of writing, a simple literature search of "synaesthesia advantages" yields 7020 results in Google Scholar, while "synaesthesia disadvantages" returns a mere 1530 hits.

However, there is a small, yet growing, body of literature that suggests synaesthesia may be associated with more clinical conditions. Much of this evidence comes from case studies of individual synaesthetes (e.g. Hänggi et al., 2008; Blakemore et al., 2005) which makes it difficult to draw conclusions about both how synaesthesia and other conditions might be linked, and if indeed any co-occurrence would be an indication of anything meaningful, such as a common neural or genetic mechanism. Recent

research has become more broad, by moving from case studies of individuals to examining comorbidity in larger samples of synaesthetes and other patient groups. This has been tackled thus far in one of two ways; either by examining the prevalence of synaesthesia in a group of patients with a particular condition, or by examining the prevalence of a particular condition in a group of synaesthetes.

The primary aim of this thesis is to investigate synaesthesia and its comorbidity with other conditions. Prior to presenting any empirical work , this chapter summarises all existing literature which suggests links between synaesthesia and any other clinical conditions. I then explore whether directly comparing healthy and clinical populations is a valid methodological approach. Below, I consider the following seven possible comorbidities; anxiety disorder, autism (and autism spectrum disorders), epilepsy, irritable bowel syndrome (IBS), migraine, multiple sclerosis and schizophrenia. These conditions are discussed in alphabetical order, rather than order of importance.

2.2 Synaesthesia and comorbidity

2.2.1 Synaesthesia and anxiety disorder

Anxiety disorder is a prevalent condition characterised by the experience of long term generalised worry and anxiety (Evans et al., 2008). Commonly regarded as belonging to a spectrum of mood related disorders, symptoms include feelings of threat, irritability and tension, lifetime prevalence is estimated to be between 4.3-5.9% of the population (Tyrer and Baldwin, 2006).

While conducting a questionnaire based study into the personality profile of synaesthetes, Banissy et al. (2012a) found higher rates of positive schizotypy, a personality trait closely linked to problems with decision making and social anxiety. In their study, 30 participants were randomly selected from an existing database of synaesthetic sub-

jects. These synaesthetes experienced colour as their concurrent sensation and had the genuineness of their synaesthesia objectively assessed by completing the Synesthesia Battery at www.synesthete.org (Eagleman et al., 2007). Participants were subsequently given the Oxford-Liverpool Inventory of Feelings and Experiences, which can reliably measure schizophrenia-like symptoms in the general population (Mason and Claridge, 2006). Given that anxiety is a key aspect of postive schizotypy (Debbané et al., 2009; Lewandowski et al., 2006), their findings imply that synaesthetes may be more likely to experience anxiety than non-synaesthetes. The authors conclude that the presence of synaesthesia may be an indication of a broader phenotype, with synaesthetes exhibiting atypical personality and cognitive profiles. The discovery of higher rates of positive schizotypy in synaesthetes (Banissy et al., 2012a) and the link between positive schizotypy and anxiety, would imply that rates of anxiety disorder may also be higher in synaesthetes. I investigate this empirically in Chapter 9.

Although participants were selected at random in the study by Banissy et al. (2012a), subjects' initial contact with the research team was via self-referral. It is therefore possible that synaesthetes who had the motivation to contact researchers and participate in research studies may exhibit different personality traits from those who do not. However, if the control subjects were recruited by the same means, this bias could reasonably be expected to be controlled for. Despite this potential confound, their study is a valuable first step in showing that synaesthetes may exhibit anxiety linked personality differences that differ significantly from non-synaesthetes. Interestingly, Banissy et al. (2012a) suggest that the presence of synaesthesia may be a marker for differences in cognition that are not solely limited to the experience of synaesthesia. The authors point out that both synaesthesia and positive schizotypy have both been linked to creativity and mental imagery vividness and point out that a disturbed balance of cortical excitation and inhibition has been put forward as a potential mechanism underlying both synaesthesia (Grossenbacher and Lovelace, 2001) and schizotypy (Grossberg,

2000). In this thesis, I will measure the prevalence of anxiety disorder in a large, random sample of the population and compare the prevalence of anxiety disorder in synaesthetes and non-synaesthetes. Because self-referral bias will be minimised in my own study, I will be able to ascertain whether anxiety disorder is more prevalent in synaesthetes, without the possible confound of self-referral bias.

2.2.2 Synaesthesia and autism spectrum disorders

Autism spectrum disorders (ASDs) are a group of neurodevelopmental conditions characterised by a high degree of aetiological and clinical heterogeneity, the behavioural hallmarks of which are impaired social function, communication difficulties and patterns of repetitive behaviours (e.g., Lauritsen, 2013).

Tentative suggestions that synaesthesia and autism may be linked have featured sporadically in the academic literature for several decades (e.g., Wohlfarth, 1985; Cytowic, 1995). Initial investigations involving autism and synaesthesia focussed on case-studies of individuals affected with both conditions (Baron-Cohen et al., 2007). For example, several such studies have involved DT, a remarkable individual with synaesthesia, Aspergers and savant abilities, who shows extreme numerical memory and ability for mathematical calculation. Baron-Cohen et al. (2007) proposed that when synaesthesia and autism co-occur, the likelihood of savantism also being diagnosed increases (also see Simner et al., 2009b; Bor et al., 2008).

Case studies such as DT had led to some speculation that synaesthesia and autism may be related (Baron-Cohen et al., 2007). However, looking to case-studies for convincing evidence of links between synaesthesia and other conditions is problematic. There is a risk of falling prey to the *illusory correlation*, whereby a relationship is perceived to exist between two rare variables which have coincidentally co-occurred, when in fact no relationship exists at all (Chapman and Chapman, 1969).

In 2013, the first empirical group studies investigating the prevalence of synaesthesia in ASD populations were conducted. In a study looking at the prevalence of synaesthesia in autism, Baron-Cohen et al. (2013) report that the prevalence of self-declared synaesthesia in a sample of 164 subjects with autism (a large majority having Asperger syndrome; N=153) was significantly higher than in a group of matched controls. This study relied predominantly on self-declaration (i.e., subjects coming forward to declare they have synaesthesia) and the authors report having difficulty in achieving compliance in tests of genuineness, indicating a lack of objective consistency testing. Objectively verifying the genuineness of cases of self-declared synaesthesia is an important step in the methodology of identifying synaesthetes. Given that the prevalence of synaesthesia can drop significantly once validation takes place (i.e., Simner et al. (2006b) report finding 1 genuine synaesthete for every 6 subjects self-declaring as experiencing synaesthesia), estimating prevalence based on self-declaration alone is likely to be inaccurate.

In another study, Neufeld et al. (2013) assessed the prevalence of grapheme-colour synaesthesia in a sample of Asperger syndrome patients. Although the sample of patients examined was small (N=21), the assessment of synaesthesia was rigorous, with both objective tests of consistency and subject interviews conducted. To assess genuineness of synaesthesia, the authors used their own version of The Synesthete Battery (Eagleman et al., 2007). A questionnaire was then given to assess the participant's subjective experience of the consistency and vividness of their experience during the test for genuineness. They found a significantly higher prevalence of grapheme-colour synaesthesia in the sample of Asperger syndrome patients (5/21 tested subjects or 23.8%), in comparison to the rate in the general population of 2% reported by Simner et al. (2006b).

There is also evidence that does *not* lend support to the hypothesis of an association between synaesthesia and autism. While a common behavioural characteristic

of autism is a reduction in empathy (Baron-Cohen and Wheelwright, 2004), there is no evidence to suggest empathy is reduced in synaesthetes. Banissy et al. (2013) conducted an investigation into personality traits of grapheme-colour synaesthetes and report that while synaesthetes may have atypical personality characteristics in some domains (e.g., openness to experience), empathy scores appear consistent with those found in demographically matched controls. Indeed, certain measures of empathy (the emotional reactivity aspect of the empathy quotient questionnaire) has been shown to be significantly *higher* in mirror-touch synaesthetes (who experience tactile sensation on their body in response to seeing another person being touched), relative to control subjects (Banissy and Ward, 2007).

Despite this discrepant finding regarding empathy, the study by Neufeld et al. (2013) does appear to show evidence that synaesthesia and autism spectrum disorders may be linked. If there is an association between synaesthesia and autism, it is possible that the link may be mediated by, or connected to, other traits. Absolute pitch (AP) - the ability to recognize the pitch of a musical note without a reference point (Deutsch, 2012) - may be one such potential trait, having been suggested as a potentially unusual connection between autism and synaesthesia. Gregersen et al. (2013) report a genetic overlap between absolute pitch and synaesthesia, and previous reports show absolute pitch to be found more frequently in a sample of autism patients (DePape et al., 2012). There is also evidence that patterns of connectivity are altered in AP, with hyperconnectivity in the superior temporal lobe reported by Loui et al. (2011). If an association between AP, synaesthesia and autism can be convincingly demonstrated, it is possible that isolating the genetic origins of a distinct cognitive trait such as absolute pitch may help to identify the genetic origins of other conditions that are more phenotypically heterogeneous.

2.2.3 Synaesthesia and epilepsy

Epilepsy is a common neurological condition characterised by seizures which has an estimated prevalence of 3.3-7.8 per 1000 people in Europe (Forsgren et al., 2005). Head trauma, other brain conditions or pre-natal injury are known risk factors for epilepsy but more than 50% of epilepsy cases have no known cause (Forsgren et al., 2005). Evidence linking synaesthesia and epilepsy is currently sporadic and largely anecdotal. Synaesthesia has been reported to be more common in patients with temporal lobe epilepsy (Ramachandran and Hubbard, 2001) but this evidence is limited to a small number of case studies in which the individuals concerned often exhibited a complex range of other comorbid conditions, or have previously experienced significant brain trauma. There is also the risk of illusory correlation in this situation where single case studies are investigated - making the assumption that because two relatively rare traits or diseases are present, they must be somehow related.

The majority of the comorbidity literature refers to synaesthetes who experience developmental synaesthesia, meaning that synaesthesia is generally present in the individual prior to the appearance of the comorbid condition. With respect to synaesthesia and epilepsy, one of the few reported cases of individuals with both conditions refers to the development of acquired synaesthesia after significant brain insult. Halligan et al. (1996) report a case study of a patient who developed a phenomenon resembling touch synaesthesia after having two major epileptic episodes subsequent to a stroke. The subject was able to reliably detect the feeling of touch on his left arm and hand, but only when able to see it. When vision was restricted, no feeling of touch was reported. The authors themselves question whether the patient's experience can be considered synaesthesia in its truest sense. However, the intrinsic nature of the experience is cross-modal, which warranted its inclusion here. It is important to draw attention to the fact that in this case, the synaesthesia is acquired, rather than developmental. It remains an open question how similar these phenomena are. Behaviourally, they appear closely

related but their origins are obviously very different (see Sinke et al., 2012; Brogaard, 2013).

Jacome (1999) reports a case study of a patient who was diagnosed with both epilepsy and multiple sclerosis, and experienced a range of unusual visual hallucinations. For example, the subject could voluntarily induce visual hallucinations of people who spoke to her or objects that made noises. Although there is an element of cross-modality to these experiences (sounds triggering visual experiences), and the author uses the term synaesthesia to describe them, it doesn't appear that they would be considered synaesthesia as it is currently understood. This is because synaesthetic associations are generally considered to occur automatically, rather be induced voluntarily, as was the case in this particular person. The subject (participant DT) of a case study published by Baron-Cohen et al. (2007), which focussed on exploring a potential link between synaesthesia, autism and savantism (see previous section) also suffered from epilepsy at age 3. The fact that the subjects in all three of the case studies discussed in this section suffered from multiple comorbidities or had experienced serious brain trauma highlights the difficulty in drawing conclusions about whether synaesthesia may be linked to other conditions, or exactly how that might have arisen.

Further evidence that epilepsy and synaesthesia might perhaps be considered in investigations of comorbidity comes from a report that epilepsy medication has also been shown to lessen synaesthetic experience in at least once instance (Cytowic, 1995). In addition, Asher et al. (2009) conducted the first genome wide study into the genetics of synaesthesia and put forward several genes linked to epilepsy as candidates worthy of further investigation. These genes - previously known to be linked to epilepsy (Escayg et al., 2000; Fujiwara et al., 2003) are located in the region of chromosome 2 identified by Asher and colleagues as most significantly linked to synaesthesia in their investigation. However, the area of chromosome 2 implicated in this study are

large and contain hundreds of genes, so no valid conclusions can be drawn linking the genetics of synaesthesia and epilepsy from this investigation.

2.2.4 Synaesthesia and irritable bowel syndrome (IBS)

Irritable bowel syndrome is a functional disorder of the gastrointestinal tract, characterised by symptoms such as abdominal bloating, constipation and diarrhoea (Thompson et al., 1999). There is also evidence to suggest that IBS patients may experience general hypersensitivity to a range of sensory stimuli (Carruthers et al., 2012).

To date, a single population based study has been conducted, and this reported a higher prevalence of synaesthesia in IBS patients (Carruthers et al., 2012). The authors limited their investigation to two forms of synaesthesia, grapheme-colour and music-colour/shape synaesthesia, and found the self-declared prevalence of synaesthesia in a group of 200 IBS patients was 13%, compared to 3% in a group of controls. Once synaesthesia was verified using a longitudinal test of consistency with a 3 month test-retest interval (i.e., completing the same test twice, three months apart), significant differences between groups still remained. Using a consistency threshold of 50% correct (reporting the same colour association with the same grapheme on both tests for at least half the stimuli), the prevalence in the IBS group was reduced to 9.5% in patients and unchanged from 3% in controls. When a stricter threshold of 75% accuracy was used, prevalence dropped to 7.5% in the IBS group and 2.5% in controls. In other words, when stricter thresholds of consistency were applied in order to verify the genuineness of the synaesthesia (from an initial 50% correct to 75% correct), the prevalence decreased by a greater degree in the patient group, whereas the prevalence found in the control group remained relatively constant. This is noteworthy, and implies that the IBS group were either more likely to report having synaesthesia when they didn't, their synaesthesia was less consistent than the control group or they experienced some difficulties in completing the test. The broader issue of comparing

clinical populations with control groups is a significant one, and is discussed in greater detail in the conclusion to this thesis (see Chapter 10).

2.2.5 Synaesthesia and migraine

Migraine is a neurological disorder characterised by unilateral throbbing headache (Bashir et al., 2013) experienced by approximately 12% of the adult population in the USA (Lipton et al., 2007). In common with the conditions discussed above, research linking migraine and synaesthesia is largely limited to case studies of individuals, limiting any conclusions that can be drawn regarding any generalised link between the two conditions. Synaesthesia has been reported to occur as a symptom of migraine (Podoll and Robinson, 2002) in a single individual, reported anecdotally to her general practitioner. A second case study detailed an individual who reported synaesthetic experiences which occurred sporadically during the aura phase of migraine. Approximately 40% of migraine sufferers experience this phase, a principal component of which are visual disturbances such as the perception of flashes of light, loss of vision or visual hallucination. In this particular case study, the synaesthetic experience took one of two forms, either visual disturbances triggered by high pitch sounds, or a lemon taste triggered by bright light (Alstadhaug and Benjaminsen, 2010). This case was complicated by the presence of an additional co-morbidity in the form of bipolar disorder. The interesting point about this example is the fact that it appears to be temporary acquired synaesthesia - a relatively rare phenomenon. It is perhaps noteworthy to mention that both cases reported here are of individuals whose professions involve working closely with colour, namely art teacher and graphic designer respectively. Synaesthesia has been shown to be more common in artistic groups, with Rothen and Meier (2010) reporting a prevalence of grapheme-colour synaesthesia of 7% in art students, significantly higher than their baseline prevalence of 2% in a sample of matched controls.

A population based study conducted in order to investigate neuropsychological symptoms associated with migraine found significantly higher prevalence of self-declared synaesthesia in migraine sufferers, with 19% prevalence versus 7% in controls (Jürgens et al., 2014). Patients who experienced migraine with auras were particularly affected when compared to migraine-without-aura sufferers, with 29% of this former group reporting synaesthesia, versus 15% of migraine-without-aura patients. This study was entirely questionnaire based, the subtype of synaesthesia experienced was not reported and no validation of the synaesthetic experience appears to have taken place. As noted above, given that the prevalence of synaesthesia can drop significantly once validation takes place (i.e., Simner et al. (2006b) report finding 1 genuine synaesthete for every 6 subjects self-declaring as experiencing synaesthesia), the absence of this critical step in this study makes drawing firm conclusions more difficult. However, the sample taking part in this study was large and considerable care had been taken to select a matched control group (219 migraineurs and 161 age and sex matched controls). Furthermore, the study examined a large number of traits that may be associated with migraine. There was no *a priori* information provided to participants about links to synaesthesia which would suggest the study would not be particularly attractive to synaesthetes. Taken together, this provides good support for the hypothesis that migraine and synaesthesia may be linked. However, only a subset of 44% of those initially contacted responded with a completed questionnaire, introducing a response bias whereby only participants who were particularly motivated to return questionnaires (i.e., perhaps the synaesthetes themselves) were included in the study.

2.2.6 Synaesthesia and multiple sclerosis

Multiple sclerosis (MS) is a disease of the central nervous system (CNS), characterised by demyelination, gliosis of white matter and axonal loss (Weiner, 2009). It is caused by a complex interaction of environmental factors coupled with genetic susceptibility (Compston and Coles, 2008). Comorbidity between multiple sclerosis and synaesthe-

sia will be a particular focus of this thesis. I first review the literature for any prior evidence of an association between synaesthesia and MS and show that prior to the study conducted here, little research has been done in this area.

Synaesthesia-like phenomena are not unusual in cases where lesions or demyelination of the optic nerve are present, and so they are a common occurrence in a majority of MS cases (Evangelou et al., 2001). Jacobs et al. (1981) reported a group of nine patients who self-declared experiencing coloured phosphenes induced by sound, during clinical interviews with their health care providers. All nine patients had white mater lesions on the optic nerve, and three of the nine patients had a diagnosis of MS. The authors propose these phosphenes occurred as a consequence of lesions to the optic nerve. A previous investigation also reported cases of MS patients who experienced similar visual disturbances (Davis et al., 1976). Two patients initially volunteered the information they experienced phosphenes induced by eye movement. Seven additional patients were subsequently questioned and were found to experience movement induced phosphenes thought to arise as a result of damage to the optic nerve. Unfortunately, no information was provided about MS patients who were questioned and did not experience such phenomena. Taken together, these findings suggest that the demyelination which is characteristic of the neurology of MS may increase the likelihood of experiencing synaesthesia, or similar cross-modal sensations.

Sensory disturbances are a common symptom of MS (Compston and Coles, 2008). For example, disturbances in the perception of vibration (Heijenbrok et al., 1991) and temperature (Leocani et al., 2003) are often reported, and altered sensations such as "pins and needles", numbness and dysaesthesia are not unusual (Miller and Leary, 2007). Particularly common are visual disturbances, present at onset in approximately 25% of MS cases (Confavreux et al., 2003).

Since some studies have described synaesthesia (or at the very least used the term

"synaesthesia") to describe some of the sensory disturbances associated with Multiple Sclerosis, it is important here to clarify two ways that synaesthesia and MS might be linked. First of all, synaesthesia might be implicated as a consequence of MS: i.e., that lesions caused by MS have knock-on effects in the sensory domain that can come to resemble synaesthesia in some way. In this case, the synaesthesia would be considered acquired. Alternatively, synaesthesia might be implicated as comorbid with MS in that both would develop in parallel in some way, or at the very least, that synaesthesia would exist prior to the onset of MS. In this case, the synaesthesia in question would be the developmental variant. It is important to clarify henceforth that this thesis is interested only in the latter type of comorbidity: it explores the possibility that developmental synaesthesia may exist prior to MS.

Given the distinction in the paragraph above it is important to here clarify how we would tell apart acquired and developmental synaesthesia, so we can consider only the latter in combination with MS. It has been suggested that developmental and acquired synaesthesia differ in the nature of their triggers: the former is triggered in the overwhelming majority of cases by linguistic stimuli (Simner, 2007) while the latter appears to be triggered more by low-level sensory stimuli. Therefore, by considering whether MS co-occurs with a prototypical linguistic synaesthesia (e.g., grapheme-colour synaesthesia) we can establish whether it co-exists with a developmental variant. Put differently, we can attempt to rule out the possibility that the synaesthesia is nothing more than a symptom of the MS, *per se*.

2.2.7 Synaesthesia and schizophrenia

Schizophrenia is a complex mental disorder, characterised by cognitive, emotional and behavioural deficits, and has a lifetime prevalence of 4.0 per 1,000 people (Saha et al., 2005). Cortical hyperconnectivity is hypothesised to play a significant role in schizophrenia (Krishnadas et al., 2014; Ford et al., 2014), providing theoretical

grounds to explore whether there might be an association with synaesthesia. Indeed, in suggesting a model linking serotonin receptor 2a (S2a) to some types of synaesthesia, Brang and Ramachandran (2008) made the prediction that the prevalence of synaesthesia would be higher in schizophrenia patients. In other words, if serotonin receptors play a key role in synaesthesia, it is a reasonable prediction that conditions also affecting serotonin might be more common in synaesthetes. Evidence that antipsychosis medications also act as serotonin receptor antagonists (Meltzer and Massey, 2011) provides further rationale to explore this hypothesis in greater depth.

Cross-modal experiences occurring in schizophrenic patients were first described in the late 1950s (Chapman et al., 1959; Freeman and McGhie, 1957). These experiences – catatonic episodes induced by auditory and visual stimulation – were interpreted by the authors as synaesthetic in nature, given the production of a motor response (catatonia) as a direct response to auditory and visual disturbance. Given the frequent clinical presentation of auditory and visual hallucinations in schizophrenia (Bauer et al., 2011), it is not difficult to imagine reports of synaesthetic experience being interpreted as symptomatic of psychiatric illness. Indeed, early descriptions - now defunct - suggested synaesthesia as being closely related to a range of psychiatric conditions and to lie somewhere on a continuum between normal perception and hallucination (see Ostwald, 1964 for discussion). Whether hallucinations could be considered similar to synaesthesia at all is a matter of interpretation. On one hand, there is often a cross-modal aspect to hallucinations that suggests the phenomena may be related. On the other, hallucinations occur seemingly at random, whereas a synaesthetic experience relies on a consistent inducer to act as stimulus (Fitzgibbon et al., 2012). Furthermore, synaesthetes know the difference between their synaesthetic experiences and reality, further emphasising the belief that synaesthesia should not be considered a psychiatric condition or a hallucination (Ward, 2013).

More recently, Banissy et al. (2012a) conducted a questionnaire based study - as

noted above - aimed at investigating the personality profile of synaesthetes. The instrument used in the study - the Oxford-Liverpool Inventory of Feelings and Experiences - measures sub-clinical schizophrenia-like symptoms in the general population (Mason and Claridge, 2006). The authors report an increase in levels of positive and disorganised schizotypy in people who experience forms of synaesthesia with colour as the concurrent sensation, and conclude that having synaesthesia may be indicative of an atypical personality profile, beyond the presence of synaesthesia itself. I discussed positive schizotypy with reference to anxiety disorder, but because positive schizotypy is associated with an elevated risk of developing schizophrenia (Barrantes-Vidal et al., 2013), the increased prevalence of schizotypy in synaesthetes may suggest an association between synaesthesia and schizophrenia.

With the aim of developing an experimental paradigm to test schizophrenic patients for digit-colour associations, Sarhan et al. (2008) tested a group of 11 schizophrenia sufferers, the majority of whom reported seeing coloured digits when in fact all presented digits were white. No repeat testing was conducted, so it is unclear whether these associations were stable and consistent over time, as would be required for a full synaesthesia diagnosis. The authors employed convenience sampling (i.e., testing patients who happened to be attending the clinic), and it is unclear whether participants were informed whether the study was about synaesthesia prior to taking part. If so, this may lead to the sort of self referral bias discussed elsewhere in this thesis (see Chapters 1, 6 and 10).

On account of the proposed similarity in altered connectivity found in schizophrenia and synaesthesia, Tomson et al. (2011) speculate that synaesthesia may be an ideal proxy by which to study altered connectivity in pathological conditions. In an investigation into the genetics of synaesthesia, they report a region on chromosome 16 that may be linked to synaesthesia and put forward a candidate protein, go-alpha (GNAO1) in this area that is under-expressed in schizophrenia patients. Linking synaesthesia

and schizophrenia via this mechanism is highly speculative, because the area of the genome identified by Tomson et al. (2011) contains many hundreds of genes, and the sample size is small for a study of this nature.

A recent case study involving a patient with an arachnoid cyst in the temporal lobe, who experienced both ticker-tape synaesthesia (the automatic visualisation of words in the style of subtitles or teleprompter; Chun and Hupé (2013)) and psychotic episodes, leading to the conclusion that lesions in this area of the brain may be linked to the development of both conditions in this patient (Bastiampillai et al., 2014). Interestingly, the patient reported that the intensity of his synaesthesia increased over time, which the authors speculate may be linked to the increasing growth of the cyst. However, the patient recalled being aware of synaesthesia aged 6, but the cyst was not diagnosed until the patient was sixteen years of age. It is likely therefore, that the presence of synaesthesia in this patient pre-dated the development of the cyst. In any case, the use of a single case study again raises the possibility of illusory correlation, and the group study reported above (Sarhan et al., 2008) did not use a robust method of identifying genuine synaesthesia.

2.2.8 Conclusions

In this chapter, I summarise the current research into synaesthesia and its comorbidities and highlight some of the issues surrounding this nascent field. The suggestion that associations may exist between synaesthesia and a variety of other conditions has been a feature of the synaesthesia literature for a considerable length of time, and there are oft-repeated statements that synaesthesia is linked to a variety of conditions. To date, the empirical evidence underlying such beliefs is often limited by a paucity of published data, and is often speculative and anecdotal. Early studies almost exclusively focus on case studies of individuals who have synaesthesia and one or more other conditions (reports linking synaesthesia to autism, epilepsy, migraine and schizophrenia fall into

this category). Predicting associations between conditions based on a small number of case studies, makes falling prey to the notion of an *illusory correlation* a distinct possibility. If an individual exhibits two traits that are rare in the general population, it is tempting to assume they must be causally linked in some way whereas in reality, their joint presence may be entirely coincidental or linked to a third factor.

Recent research has attempted to take a more empirical view and examine the possible relationships between synaesthesia and a broad range of other conditions on a group level (group studies have been carried out linking synaesthesia to anxiety (indirectly via positive schizotypy), autism, IBS, migraine, MS and schizophrenia). As the first attempts at this kind of study show, the methodological difficulties associated with this approach can be manifold. The prevalence of the conditions discussed above and synaesthesia in the general population are typically low, making it a difficult and time consuming process to recruit and test populations large enough to enable meaningful statistical conclusions to be drawn. The question of how to make meaningful comparisons between groups of healthy synaesthetes and synaesthetes with clinical conditions is also a significant one, which I discuss further in the conclusions to this thesis (see Chapter 10).

How might this area of synaesthesia research be advanced? A solution to these problems lies in conducting suitably sized, well designed group studies. In this thesis, I conduct one such study, examining the prevalence of synaesthesia in a sample of multiple sclerosis patients (see Chapter 8). Prevalence studies alone are unlikely to shed much light onto the underlying causes of synaesthesia. They are however, an important step in the process, and insight into the origins of synaesthesia may emerge if comorbidities are found with conditions for which the causes are already better understood. It is also worth placing the field of synaesthesia and comorbidity research in a broader context. What are the implications of identifying associations between synaesthesia and other conditions? Firstly, associations between conditions may help

to shed light on the causal roots of the conditions under investigation. Secondly, it is possible that the presence of synaesthesia in an individual may provide information regarding their risk of developing conditions in later life, assuming that associations between those conditions and synaesthesia prove to be robust. Because synaesthesia is considered to be neurodevelopmental in nature and many of the conditions thought to be associated with synaesthesia first occur at a later age, having synaesthesia may assist in understanding whether a person has an increased risk of developing a certain condition when they are older. Clearly, there are many factors which contribute to the development of a disease, but the presence of synaesthesia may contribute additional evidence regarding these risks.

In the next chapter, I examine the relationship between synaesthesia and multiple sclerosis from a different perspective, by presenting data demonstrating that multiple sclerosis and clinical precursor conditions, appear to be more prevalent in synaesthetes self-referring to participate in scientific studies than in the general population.

Chapter 3

Prevalence of multiple sclerosis in synaesthesia

3.1 Introduction

In chapter 1, I introduced synaesthesia and briefly discussed its development, its causes and the issues surrounding the identification and testing of synaesthesia in the research environment. In chapter 2, I reviewed the literature regarding synaesthesia and its potential comorbidities. In this chapter, I discuss one possible comorbidity in particular: multiple sclerosis (MS). First, I present a brief introduction to multiple sclerosis, and its precursor syndromes; *clinically isolated syndrome (CIS)* and *radiologically isolated syndrome (RIS)*. I then introduce new data showing that the shared MRI profile of MS and RIS is significantly over-represented in synaesthetes who had participated in neuroimaging research. I do this by investigating a group of synaesthetes, who have been scanned for unrelated purposes and who have shown unusually high rates of white matter hyperintensities indicative of MS and its related conditions. I present validation of the clinical and MRI status of these synaesthetes, and an analysis showing the significant probability their unusual number of MS-related cases may not have arisen by chance, given the prevalence of MS in the general population. I then review this

finding in the context of the entire published literature of MRI studies of synaesthesia to show that even when all cases of scanned synaesthetes are taken into account, the number of MS-linked cases remain statistically unexpected. I then discuss how to interpret significant data based on small case-numbers, and consider the implications of my findings for synaesthesia's clinical status. Sections of this chapter have been published in the form of a journal article, as Simner et al. (2014) (see Appendix F).

3.1.1 Multiple Sclerosis

In Chapter 2, I gave a brief introduction to MS, but I now elaborate on this overview further, given the central focus on MS in the current chapter. As noted above, MS is an inflammatory, degenerative disease of the central nervous system thought to be caused by exposure to environmental risk factors in persons with genetic susceptibility to the condition (Compston and Coles, 2008). It is strongly mediated by immune system interaction (Trapp and Nave, 2008). The condition is characterised by demyelination, axonal loss and gliosis of white matter (Weiner, 2009; Milo and Kahana, 2010; Noseworthy, 1999). The mechanisms controlling these processes are relatively poorly understood (McFarland and Martin, 2007). MS symptoms are varied and can include a range of motor, sensory and cognitive impairments that can occur independently of one another (Chiaravalloti and DeLuca, 2008). Typical symptoms can include for example, numbness, loss of sensation, pain, loss of vision, and memory deficits (Compston and Coles, 2008).

Although MS is typically classified as a white matter disorder (and I explore this in greater detail below), grey matter is also known to be affected (Geurts and Barkhof, 2008), with reduced volume common in several areas throughout the cortex of MS sufferers (Ceccarelli et al., 2008). The causes of grey matter damage are unknown and it is currently unclear whether white matter and grey matter damage occur independently or whether they are related (Raz et al., 2010).

3.1.1.1 Diagnosis of MS

In this chapter I will be considering rates of MS within populations of people with synaesthesia. It will therefore be important to understand exactly how MS is diagnosed, so that we have a clear understanding of which participants are affected. The MacDonald Criteria are a universally accepted "gold standard" set of criteria used for the diagnosis of MS (Polman et al., 2011). According to these specifications, a number of clinical criteria must be fulfilled in order for a diagnosis of MS to be given (Polman et al., 2011). The patient must have experienced a minimum of two episode of behavioural symptoms (e.g., visual disturbances, paresthesia, or walking difficulties) or a minimum of two white matter lesions in MS-typical areas of the central nervous system (e.g., brain stem, periventricular regions, spinal cord) (Compston and Coles, 2008).

If the minimum criteria above are not fulfilled, "dissemination in either space and/or time" must also be demonstrated. There are numerous ways in which these criteria can be met. Dissemination in space means either multiple sites in the body must be affected (e.g. white matter lesions in different parts of the body, such as spinal cord and periventricular regions of the cortex, or clinical episodes affecting different parts of the body, such as loss of vision and loss of limb sensation). Dissemination in time is shown by the occurrence of distinct clinical events separated by a period of time (e.g., the appearance of new white matter lesions in subsequent MRI scans, or two clinical episodes weeks or months apart). In addition to the above, other clinical diagnoses must also be excluded, such as other inflammatory disorders or infections of the central nervous system (Polman et al., 2011). If the above criteria are met, a diagnosis of multiple sclerosis is given.

3.1.2 Other syndromes linked to the development of MS

As noted above, two distinct syndromes which meet a subset of these criteria are recognised as precursors to a clinical diagnosis of multiple sclerosis; *clinically isolated syn-*

drome (CIS) and *radiologically isolated syndrome (RIS)*. Because these conditions are strongly linked to MS - most patients who eventually receive a diagnosis of MS will first be diagnosed with one of these two syndromes - both RIS and CIS will be introduced here for completeness, although it is the former in particular which arises within the sample I will be investigating.

Clinically Isolated Syndrome (CIS) is a term used to describe the presentation of the first episode of clinical symptoms indicative of a demyelinating disease such as MS affecting the central nervous system (Miller et al., 2012). Because of the relapse-remitting profile of MS (periods of illness interspersed with periods of recovery and good health), the majority of patients who eventually become diagnosed with MS will experience - and recover from - an initial clinically isolated syndrome (Confavreux and Vukusic, 2006). Not every episode of CIS will result in progression to clinically definite MS. However, between 30% and 70% of CIS patients will eventually be diagnosed with MS (Miller et al., 2005). Although a diagnosis of CIS can be given in the presence or absence of MRI abnormalities, the presence of detectable white matter lesions significantly increases the likelihood that the patient will develop MS (Fisniku et al., 2008). Furthermore, the number of lesions correlates positively with the risk of progression (Tintore et al., 2010).

Radiologically isolated syndrome (RIS) is defined as the discovery of incidental MRI findings which are suggestive of MS (namely white matter lesions), but without the person in question displaying any overt clinical symptoms (Okuda et al., 2009). It is this condition (along with MS) that will be relevant to the current study (see further below). From a radiological perspective, MRI scans of RIS and MS are indistinguishable, and it is only the presence of clinical symptoms (which appear in the latter only) that differentiates between the two conditions. RIS is uncommon, with an estimated prevalence in the general population of 0.05% (Morris et al., 2009). Because this study presents an analysis of the prevalence of MS and RIS in synaesthetes, and because RIS

is typically an incidental finding (i.e., unintentionally discovered as a result of a unrelated imaging investigation), it is important to know this prevalence of RIS in the general population and to note its rarity. Evidence linking RIS and MS however, is significant. Despite the lack of clinical symptoms, RIS patients demonstrate reduced cognitive performance akin to those of MS patients (Lebrun et al., 2010). In addition, approximately one third of patients with RIS develop symptoms characteristic of MS within 2-5 years, and RIS has been described as "preclinical MS" (Granberg et al., 2013).

3.2 Why might synaesthesia and multiple sclerosis be linked?

In this chapter I will present data showing a statistical relationship between MS and synaesthesia: I will show a higher than chance occurrence of MS/RIS in a group of synaesthetes who had been scanned using MRI (for unrelated reasons). In this section I therefore consider whether there are any other reasons to believe that MS/RIS and synaesthesia may be linked, and I begin by looking at the neurological profiles of each condition.

Synaesthesia is characterised by structural differences in white and grey matter compared to the general population and this is also a feature of MS. As discussed in Chapter 1, synaesthetes show both increases and decreases in grey matter volume and increased cortical thickness in the fusiform gyrus, frontal cortex and parietal cortex (specifically, the inferior and superior parietal lobules, anterior intraparietal sulcus and posterior cingulate gyrus; Jäncke et al., 2009; Hänggi et al., 2008; Weiss and Fink, 2009; Rouw and Scholte, 2010). Altered coherence of white matter is also reported (Rouw and Scholte, 2007; Hänggi et al., 2011; Jäncke et al., 2009), and is found in regions implicated by the synaesthetic report, and also elsewhere. Specifically, synaes-

thetes experiencing colour sensations show increased FA (i.e., increased white matter connectivity) compared with controls in and adjacent to colour selective region V4 (Rouw and Scholte, 2007; Jäncke et al., 2009) but also show FA increases (Rouw and Scholte, 2007) and decreases (Hänggi et al., 2008) in the parietal lobe. In addition to this, altered white matter connectivity has been reported in frontal (Hänggi et al., 2008) and temporal (Rouw and Scholte, 2007) regions. The cortical reorganisation of white and grey matter in synaesthesia might call for comparison with MS/RIS, since I ask in this chapter whether epidemiological or pathological links might exist between them.

Grey matter pathology in MS can be widespread and diffuse, potentially affecting all areas of the brain (Geurts et al., 2012). White matter damage can also be widely distributed in MS (Kutzelnigg et al., 2005). In particular, both grey and white matter, pathology has been found in the frontal, parietal and temporal lobes (Bø et al., 2003; Kutzelnigg et al., 2007; Sbardella et al., 2013) and these same lobes show changes in synaesthesia too (see above; Hänggi et al., 2008; Jäncke et al., 2009; Rouw and Scholte, 2007; Weiss and Fink, 2009). Given the widespread overlap of affected areas in both MS and synaesthesia, it is possible therefore, to identify brain regions that are implicated in both MS and synaesthesia. Specifically,there is evidence for changes in both MS and synaesthesia in the following regions: fusiform gyrus (Datta et al., 2015; Jäncke et al., 2009) and parietal lobe (Miller et al., 2003; Rouw and Scholte, 2010). Superficially at least, it therefore seems as if the two conditions can involve similar neurological profiles, when considered at this level.

I suggest here however that this level of comparison is probably unhelpful in any attempt to link MS and synaesthesia. Although it is true that both conditions cause white/grey matter changes, and although some of these changes can arise in the same or neighbouring regions, I suggest that this alone is not sufficient to draw any strong conclusions about comorbidities between the two conditions. One reason for this is that similar profiles are also seen in a range of other conditions. For example, although

grey matter volume changes in parietal lobe have been noted in synaesthesia, changes in the parietal lobe have also been reported in a wide range of other conditions, such as schizophrenia (Job et al., 2005), epilepsy (Bernasconi et al., 2004) and Parkinson's disease (Melzer et al., 2012). Similarly, altered coherence of white matter in the brain regions implicated above in synaesthesia has been shown in a range of conditions, such as autism (Mueller et al., 2013), schizophenia (Roalf et al., 2015) and anxiety (Baur et al., 2011). For this reason we must be careful to not draw strong conclusions based on this type of neurological similarity alone - although clearly having two conditions that alter coherence in white and grey matter is a reasonable place to start for comparison.

The key reason to draw a parallel between synaesthesia and MS is found in the data I will present below. This question of an association between MS and synaesthesia is motivated below by a finding of particularly high occurrence of MRI abnormalities consistent with the neuroradiological profile of MS/RIS within synaesthetes who have presented to our laboratories. I present these data below.

3.3 Experiment 1

In this study I consider three independent synaesthete cohorts tested in three different countries (San Diego, USA; Paris, France; Edinburgh, UK). After these studies were complete, my research group (Prof. Julia Simner and Dr. Ed Hubbard) opportunistically noticed there were three separate pathological cases among the 29 synaesthetes scanned, and that all three cases were related to MS. Specifically, all three showed the clinical and/or radiological indicators suggestive of MS in 1 in 6 synaesthetes (San Diego; Hubbard et al., 2005), 1 in 10 synaesthetes (Paris; unpublished data) and 1 in 13 synaesthetes (Edinburgh; Simner et al., in review), respectively. In other words, three affected participants were identified in a sample of 29 subjects in total. These studies were not initiated with the intention of investigating the prevalence of MS/RIS,

but these unexpected findings led us to further evaluate this post-hoc detection of white matter abnormalities in these three participants. Because the participants recruited to take part in these three studies were regarded as being in good general health, and because the association between MS and synaesthesia investigated by this study was not yet identified at the time at which these participants were recruited, information about presence of other conditions known to be comorbid with MS was not requested from participants during the recruitment phase. Below, I will show that all three affected cases met the McDonald (imaging) criteria for the diagnosis of MS, namely multiple white matter hyperintensities spread throughout the cortex. At the time of writing this thesis, Subject 1 (USA) has a full diagnosis of MS, while Subjects 2 (France) and 3 (UK) are currently free from clinical symptoms and therefore considered to have RIS. Subjects 2 and 3 were initially flagged by routine protocols in these studies in which neuroradiologists examine T2-weighted axial MRI scans for unanticipated pathology. Subject 1's diagnosis was first suggested by her GP several months after being tested by Hubbard et al. (2005), because routine radiological checks were not part of that study's protocol. Below I give detailed case studies of each subject, which I have been able to generate for the purpose of this thesis.

3.3.1 Case-details of affected synaesthetes

Synaesthesia Status: Table 3.1 shows the synaesthetic status and demographic background of the three synaesthete participants in whom radiological anomalies have been identified. The table shows their synaesthesia phenotypes, and these comprise the following: *grapheme-colour synaesthesia* gives rise to coloured percepts triggered by letters and/or digits (e.g., the letter A might be red; Asher et al., 2006); *sequence-personality synaesthesia* gives rise to complex personifications triggered by the members of linguistic sequences (e.g., Monday might be female, unfriendly; Simner and Holenstein, 2007); *number-space synaesthesia, letter-space synaesthesia* and *time-*

space synaesthesia are all variants of the broader category of sequence-space synaesthesia, in which linguistic sequences are perceived in spatial arrays (e.g., the letters A-Z might extend in an undulating line from right-to-left across the visual field; see Simner, 2009 for review).

Test Site	Age	Sex	Nationality	Synaesthesia Phenotype(s)*	MS-RIS?
San Diego	26	female	American	grapheme-colour	MS
Paris	25	female	French	number-space, time-space (coloured), grapheme-colour	RIS
Edinburgh	31	female	British /English	sequence-personality, letter-space, numbers-space, time-space	RIS

Table 3.1: **Synaesthetic case descriptions. For each affected synaesthete, the table shows the imaging lab testing site, the participant's age at scanning, sex, country of origin (nationality), verified synaesthesia phenotype(s), and the clinical status.**

* As discussed in Chapter 1, verification of synaesthesia relies on the behavioural gold standard test for synaesthesia, which assesses the consistency of the synaesthetic report over time (e.g., Simner et al., 2006b; Eagleman et al., 2007). In this, participants are required to first report their synaesthetic experiences for a list of provided stimuli (e.g., they report their colours for a list of letters), and they are subsequently retested after some considerable time (e.g., 6 months, for the case scanned in San Diego; see Hubbard et al., 2005 for methodological details). Synaesthetes are identified as those who are significantly more consistent in their reports over time, compared to a group of matched controls who invent/recall analogous associations.

3.3.1.1 Clinical status of MS/RIS

In this section, I next evaluate our three cases of MS/RIS both qualitatively and quantitatively, beginning with a detailed clinical evaluation.

3.3.1.2 Subject 1 (San Diego, USA)

This subject was diagnosed with clinically definite relapsing-remitting MS several months after taking part in a synaesthesia study in 2001. A diagnosis of MS was first suggested by this participant's GP because routine radiological checks were not part of that study's protocol. Subsequent neurological follow-up consisting of sagittal and axial T1 FLAIR, axial T2 and axial and coronal T1 weighted MRI scans revealed

extensive hyperintensities in both the brain and cervical spine, considered to be pathognomonic for MS. These white matter lesions, in conjunction with a history of clinical symptoms, confirmed this participant's diagnosis of MS. This participant had a total of 16 lesions, in both periventricular and juxtacortical white matter (see Figure 3.1). Periventricular lesions were located in the corpus callosum and in occipital and parietal areas. Juxtacortical lesions were found in the parietal region. Lesions were also detected in the spinal cord. This subject was originally scanned for, but subsequently excluded from, Hubbard et al. (2005) (see Table 3.2).

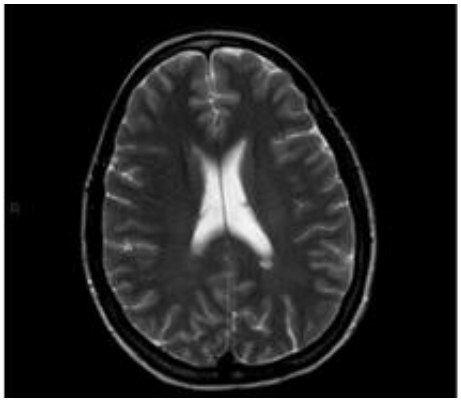


Figure 3.1: **Axial T2-weighted image showing a left parietal ovoid periventricular lesion (of 16 overall lesions), from Subject 1**

3.3.1.3 Subject 2 (Paris, France)

This subject participated in synaesthesia research in 2006, where her axial T2 MRI scan was reviewed as part of routine assessments for unanticipated pathology by a consultant neuroradiologist. Initial examination of her resultant T2-weighted MRI scan revealed white matter hyperintensities in the brain. A second, axial T2 FLAIR MRI, obtained 2 years after the initial scan, confirmed the presence of white matter lesions, judged to be consistent with the McDonald criteria (See Figure 3.2). Due to a lack of progression, these lesions are considered stable and this participant has remained free of clinical symptoms. This participant had more than 20 identified lesions in periventricular and juxtacortical white matter. Periventricular lesions were in frontal

and parietal areas, while juxtacortical lesions were distributed across frontal, parietal and temporal regions. This subject was scanned for Hubbard et al., unpublished data (see Table 3.2).

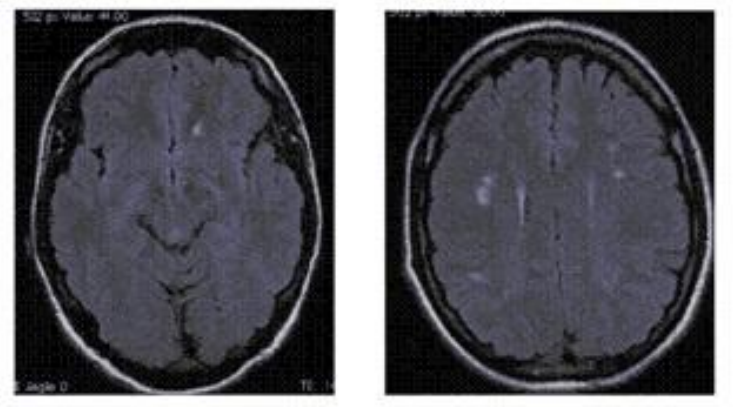


Figure 3.2: **Axial T2-weighted FLAIR images showing (left) a left frontal periventricular lesion and (right) multiple lesions; the right parietal lesion involves juxtacortical U fibres**

3.3.1.4 Subject 3 (Edinburgh, UK)

This subject participated in synaesthesia research in 2008 and her resultant axial T2 MRI scan was also examined routinely by a consultant radiologist to detect unanticipated pathology. Initial examination revealed white matter hyperintensities in the brain of this subject. This T2-weighted MRI scan was independently examined by a second neuroradiologist who confirmed that the number and location of the white matter lesions met the McDonald imaging criteria for diagnosis of MS (see Figure 3.3). Again, this participant has remained free of clinical symptoms since the initial presentation of her abnormal scan. This participant had more than 20 lesions in periventricular and juxtacortical white matter (see Figure 3.3). Periventricular lesions were found in frontal, parietal, temporal and occipital areas. Juxtracortical lesions were identified in temporal and parietal areas. Infratentorial lesions were also present. This subject was scanned for, and subsequently excluded from, a study by Simner et al., in review (see Table 3.2).

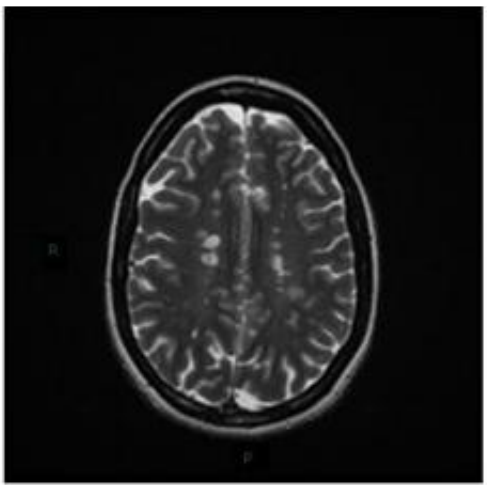


Figure 3.3: **Axial T2-weighted image showing multiple ovoid periventricular white matter lesions, from Subject 3**

3.3.2 Case-details of all synaesthetes with existing MRI scans

This finding of three cases of MS/RIS among 29 synaesthetes is suggestive of an unusually high rate. However, the possibility must be addressed that we inadvertently focussed on just those studies where anomalies were found, rather than all synaesthesia imaging studies to date. At the time this chapter was written, 29 published studies (see table 3.2) had collectively scanned 211 synaesthetes (including 6 of our own 29 synaesthetes, described in Hubbard et al., 2005). Together with our research group's remaining unpublished cases (n=23), this gives 234 synaesthete scans in existence, known to us. When conducting MRI scans for research purposes, the scan may be examined by a clinical professional for any obvious signs of pathology. Whether this procedure is followed or not depends on the policy of the research institution conducting the study. Because the 234 MRI scans I have identified were conducted in a wide range of academic institutions in different countries (see table 3.2 for details), it was not initially clear which scans had been checked and which had not. In order to obtain this information, I contacted the authors of every study in which MRI scans of synaesthetes had been recorded (see Table 3.2).

Outwith our cohort of 29 participants, I have been able to ascertain that 80 addi-

tional synaesthetes MRI scans were checked with a similar radiologist protocol, and none revealed anomalous findings of this type. A further 121 were scanned without being checked by a clinical professional and the remaining four scans have an unknown status (see Table 3.2). The most conservative approach is to assume no cases of pathology in any of the 234 scanned cases, other than the three identified here. The methodology below evaluates this occurrence of MS/RIS in synaesthetes against appropriate baselines, calculated from known prevalence rates of MS and RIS. First, I present a set of analyses which aim to establish the appropriate baselines against which to compare our finding of 3 affected cases (1 MS; 2 RIS). Below that, I will evaluate the rates of MS/RIS found in this sample statistically, both compared to the original cohort of 29 subjects and again with reference to all identified synaesthetes listed in Table 3.2. To anticipate the methodologies below, I take the most stringent standards against which to compare our observations (given factors such as the geographic variability of MS, and the sampling method of our studies) and nonetheless find that the number of affected cases is significantly higher than chance would predict.

Table 3.2 shows what is to the best of my knowledge all studies that have generated MRI scans from synaesthete participants at the time of writing including all published studies, plus our two unpublished samples. Published articles were retrieved from an all-years search of PubMed using search terms syn*esthesia (UK/US spellings) and MRI, and any additional details on participants shown below that were not available in the literature were retrieved by contact with the authors of these studies.

Year	*Authors*	*Total n*	*Status*	*Female n*
European studies				
2001	Aleman et al.	1	¥	1
2001	Weiss et al.	1	†	1
2005	Weiss et al.	9	†	6
2006	Hubbard et al. (unpublished)	10	❖	10
2006	Sperling et al.	4		4
2007	Rouw & Scholte	0*		0
2008	Hanggi et al.	1	¥	1
2009	Janke et al.	0*		0
2009	Weiss & Fink	16*	†	15
2010	Rouw & Scholte	42	¥	42
2010	van Leeuwen et al.	21	¥	19
2011	Gaschler-Markefski et al.	7	¥	6
2011	Hanggi et al.	24	¥	20
2011	Specht & Laeng	2	††	2
2011	van Leeuwen et al.	0*		0
2012	Dovern et al.	5*	†	5
2012	Hupe et al.	10	¥	7
2012	Neufeld et al.	14	¥	9
UK studies				
2002	Nunn et al.	13	††	13
2005	Blakemore et al.	1	††	1
2006	Gray et al.	2*	††	2
2007	Cohen Kadosh et al.	1	††	0
2008	Bor et al.	1	††	0
2008	Tang et al.	10	††	8
2011	Jones et al.	2	†† α	1
2012	Banissy et al	9	†	5
2013	Simner et al. (in review)	13	❖	13
N. American studies				
2003	Elias et al.	1	¥	1
2005	Hubbard et al.	6	❖	3
2012	Brogaard et al.	1	††	0
Other studies				
2006	Rich et al.	7	††	6
	TOTAL	**234**		**201**

Key

* Subject numbers have been adjusted to exclude duplicate participants already scanned in previous studies by the same group (shown elsewhere in the table)
❖Members of our study cohort (i.e., studies directed in our labs); 3 anomalies in n=29 scans
α A study directed by colleagues outside our labs but JS co-authoring
† Routine radiological checks for pathology performed by neurologist and no anomalies found
†† Routine radiological checks carried out by a neuroradiologist and no anomalies found
¥ No routine radiological checks for pathology performed

Table 3.2: **MRI studies of synaesthete participants. Table shows Year (of publication; or year of report for unpublished studies), Authors, Total n (total participant numbers), Status with respect to radiological anomalies (see key; unknown unless otherwise stated), and Female n (number of female participants).**

3.3.3 Analysis of the prevalence of MS/RIS cases among scanned synaesthetes against expected baselines

We have found three cases of MS/RIS in 234 synaesthetes who have self-referred for brain scanning studies across the literature. In order to establish whether the number of affected synaesthetes is statistically significant, I must compare the prevalence of observed cases against a meaningful baseline. Since RIS and MS are indistinguishable from a radiological perspective, I first consider all three cases as a unified phenomenon. Then, since RIS and MS are different in a clinical/symptomatic sense, I additionally consider the two cases of RIS as a distinct phenomenon. Methodologically speaking, I will calculate the RIS baseline from a study by Morris et al. (2009), which is a meta-analysis of the prevalence of RIS across all MRI scans described in the imaging literature (and below I further describe the conservative nature of my approach in this regard).

Next, I will take our MS baselines from rates reported in clinical prevalence studies. Since the prevalence of MS is sex-linked and geographically variable, I have considered that the large majority of all scanned synaesthetes are female (86%) and have come from studies conducted in Europe (94%). Accordingly, I took a female MS prevalence figure for Europe. Again however, since a key aim was to be maximally conservative (i.e., to compare our findings with the highest prevalence rates where possible), I additionally re-analysed our data using the most stringent prevalence rate according to the nationality of the three affected cases (American/French/English). In other words, since the rate of MS is highest in England (vs. America or France), I used this highest England baseline in a second analysis. Below, these baseline-selection procedures are described in more detail, along with the resultant statistical outcomes of these analyses (showing whether MS/RIS is indeed significantly higher in self-referred synaesthete samples).

3.3.4 Baseline selection and results

My first analysis below will compare all three clinical cases as a single phenomenon. Since there is no published combined prevalence of MS/RIS, I here calculate an additive value from each separately. A recent meta-analysis (Morris et al., 2009) shows a baseline of RIS in the general population of 57.8/100,000. This figure was based on nine cases of unanticipated white matter hyperintensities (WMH) considered as definite demyelination, which were found in 15,559 scans reported in the literature. To be conservative here, I will also include three cases of possible demyelination in that same meta-analysis, and furthermore, I will consider scans only from self-referred volunteer research participants (giving a total of 12 RIS cases found in 8,441 research scans; Proportion (P) =0.14%, 95% CI [0.08%, 0.25%]; Morris et al., 2009). I consider only self-referred volunteers because this type of participant is similar to those in this cohort of synaesthetes, and across the 234 scanned synaesthetes more widely. It is important to consider this method of (self-)referral since it may have elevated the number of RIS cases in our cohort. Specifically, self-referred/volunteer recruitment in any brain imaging study may increase rates of neuropathology by over-recruitment of participants who are seeking covert evaluation for undeclared neurological complaints (Morris et al., 2009). In other words, the three affected synaesthetes may have volunteered for our studies in order to assuage personal neurological concerns, and I must therefore compare their number against a baseline that specifically takes this into account.

In summary, all these considerations give us a comparative baseline for RIS in the general population of 142/100,000. However, I point out that this baseline is likely to be highly inflated since closer inspection of the data that contributed to the meta-analysis of Morris et al. (2009) reveals that two-thirds of cases contributing to this figure were found in a single study in which 90.0% of participants were former lead workers with a mean age of 60.1 years (Alphs et al., 2006). Since the likelihood of detecting white matter hyperintensities increases significantly with age (Smith et al.,

2000) and with exposure to neurotoxicants such as lead (Stewart et al., 2006), the true prevalence of white matter hyperintensities in the general population of self-referred volunteers is likely to be substantially lower than the baseline we are selecting here. Nonetheless, I use this baseline as a maximally conservative estimate (to combine with a suitable estimate for MS prevalence below), for comparison with the synaesthete sample.

The prevalence of MS in the general population is better understood, and known to vary by geographic region (with particularly high rates in Scotland; Sutherland, 1956) and by sex (females approximately 2:1; Pugliatti et al., 2006). This is of note given that one of the testing sites where MS/RIS was found was in Edinburgh, Scotland (although only four of 13 participants were Scottish; the remainder travelled from England), and also the fact that the scanned synaesthesia population is skewed towards females (F= 201). Considering the sex and nationality of the 234 scanned synaesthetes (using the location of testing centres as an indicator of nationality for cases unknown to us), an appropriate baseline is the rate of MS in female Europeans (110/100,000) since synaesthetes were virtually exclusively European (94%, n=220; including four Scots, none of whom showed pathology). This we combined with the baseline for RIS to give an additive RIS-MS baseline of 252/100,000.

Given the baseline above, I will now calculate the probability of obtaining our observed number of MS/RIS cases within scanned synaesthetes, using the exact binomial test. Thus, I estimate the probability of three observations of MS/RIS among 234 self-referred synaesthetes (Proportion (P) =1.3%, 95% binomial CI [0.3%, 3.7%]; 1282/100,000) given an expected population prevalence of 252/100,000. My statistical test shows this rate among synaesthetes to be significantly higher than chance would predict (p=.02). This difference remains significant (p=.04) even if I substitute the female Europe MS rate and use instead the female England MS rate, which is the most extremely conservative option given the demographics of our three affected cases (American/French/English).

Combining our three cases has allowed us to consider the shared MRI profile of MS and RIS, and their related clinical progression (Granberg et al., 2013). However, I might alternatively exclude our case of MS as a separate phenomenon, and consider only the two observations of RIS against all 103 scans with protocols to check for such anomaly (P=1.9%, 95% binomial CI [0.2%, 6.7%]. Taking again the highest RIS baseline (142/100,000), this finding is also highly significant (p=.01). Indeed, taking even the most extreme assumption of no other cases in any synaesthete scanned to date – checked or unchecked – an observation of two cases in 234 (P=0.9%; binomial CI [0.1%, 3.1%]) remains significant (p=.04).

3.4 Discussion

I initially observed three cases showing white-matter hyperintensities compatible with the radiological profile of MS – one case of MS and two of RIS – in 29 synaesthetes from three of our imaging labs across three different countries. I have taken the most conservative approach in assuming no further cases in any synaesthetes scanned to date, placing the prevalence at three affected cases in 234 synaesthetes (1,282/100,000, compared to a population baseline estimate of 252/100,000). Two cases of RIS in 103 brain scans checked for pathologies would, if representative, place the prevalence of RIS in synaesthetes at 1,942/100,000 (compared to a baseline of 142/100,000). These rates are significantly higher than expected, even against our highly conservative baselines. I also specifically controlled for the possibility that our three affected cases may have volunteered for our studies in order to assuage personal neurological concerns. I did this by comparing our RIS cases to a baseline constructed only from studies that included similar, self-referred, volunteer participants. In other words, I compared our rates to studies likely to have just as many concerned self-referrers as our own, rather than to studies using non-voluntary recruitment methods (e.g., work-related health screening; Weber and Knopf, 2004).

It is important to clearly acknowledge that only a small number of affected cases

are reported here, and on this basis, I do not claim that synaesthesia causes MS/RIS. Indeed, our small sample size means it would be prudent to hold back from making any strong claim whatsoever about links between these two conditions. There are many environmental factors thought to contribute to the development of MS and RIS; the evidence I present here suggests having synaesthesia may be one factor that could merit further investigation. Indeed, I have chosen to present these data for two reasons. The first is that the rates I have found are statistically significant; a considerably greater sample size would usually be needed in order to detect the numbers we have found although again our sample sizes are small. The second reason I present our data is a practical one: cases of pathology are usually excluded from MRI study populations as soon as they are detected. Therefore, they often do not appear in the literature and so remain unknown outside the research group. I report these data because it is important that other researchers working in this area are aware of these cases, so they do not overlook future neurological abnormalities, should they ever be discovered. If a meaningful link between synaesthesia and MS did exist, and as scanning of synaesthetes for research purposes becomes more commonplace, further cases of this nature would arise, and so I encourage researchers to make any cases known to the wider community if they share similarities with our own.

As noted in Chapter 2, one explanation for a link between synaesthesia and MS could be the occurrence of synaesthesia-like symptoms after the onset of MS. In other words, it is possible that the degenerative neurological damage caused by MS might give rise to sensory disorders that mimic synaesthesia, while having different causes. We saw above that sensory disturbances do accompany MS (e.g., changes in colour vision; Gregori et al., 2011) although this hypothesis would imply that the onset of synaesthesia-like symptoms should be later in life – resulting from MS-related changes in brain structure. However, I do not believe this account for the cases I present here, and for two reasons. I pointed out above that later-acquired synaesthesias are qualitatively different to developmental variants (Ward, 2013) and do not reflect the synaes-

thesias of our participants. Acquired variants of synaesthesia tend to involve low-level sensory triggers (e.g., tones) rather than learned symbols such as graphemes (Ward, 2013) although it is precisely this latter type of trigger (graphemes etc.) that our own cases possess here and which might therefore be considered a hallmark of developmental synaesthesia. Furthermore, all three of our cases report life-long synaesthesia, stemming back from early childhood, and being present for as long as they can remember.

In conclusion to this chapter, I have demonstrated a statistical link between MS/RIS and synaesthesia. Overall, I have been conservative in this study in three ways: baselines were selected in an overly conservative way; I took additional measures to be conservative when conducting my statistical analysis (e.g., assuming no anomalies in scans not assessed by radiologists); and I am circumspect in the interpretation of these findings. Because this study relies on a small number of cases - three only - I do not claim that any causal link necessarily exists between synaesthesia and MS/RIS, and I present these findings with this strong caveat. Nonetheless, if these epidemiological findings are indeed later supported by additional evidence, this could invite a debate about the clinical status of synaesthesia, which has previously been associated with largely beneficial rather than unfavourable characteristics.

In the following chapters, I will further explore this possible link between synaesthesia and MS in two additional ways. In chapter 6, I will present a study of the genetics of synaesthesia. For this, in chapter 4, I first provide a brief introduction to genetics and the methodology of genetic studies. I then review and evaluate the literature of synaesthesia genetics research, provide an overview of multiple sclerosis genetics and raise the possibility that an association between synaesthesia and multiple sclerosis may share common genetic origins. Subsequently, in Chapter 8, I will explore this potential association from the opposing direction, namely by examining the prevalence of synaesthesia in MS patients.

Chapter 4

Genetics literature review

In anticipation of my genetics investigation into links between synaesthesia, MS and immunity (see Chapter 7), in this chapter, I provide an introduction to genetics in general and an appraisal of possible approaches to conducting genetic experiments. I then review the current understanding of synaesthesia genetics and provide an overview of the genetics of MS. Finally, I put forward reasons why MS and synaesthesia may share a genetic basis, and provide the rationale for the genetics work conducted for this thesis (see Chapter 7). Sections of this chapter have been published in the form of a book chapter, as Asher and Carmichael (2013) (see Appendix D).

4.1 A brief introduction to genetics

The genome - the genetic code of an organism - is encoded by a complex molecule called DNA, stored in the nucleus of almost every cell in our bodies. DNA is stored in the form of chromosomes, which are large molecules of DNA, coiled around a specific type of protein called histones that provide structure to the molecule. DNA consists of nucleotides; each nucleotide consisting of a nitrogenous base, a dioxyribose sugar and a phosphate group. DNA is made up of four different nitrogenous bases, cytosine (denoted by the letter 'C'), guanine (G), adenine (A) and thymine (T). A molecule of DNA consists of a long string of nucleotides joined together, the nomenclature of

which is denoted by the corresponding base letter, such that a segment of DNA could be portrayed as follows, for example: A-T-T-G-A-C-C-T-A-G-C-T-A-C. The entire human genome is three billion bases in length and is approximately one metre long. Genes are specific sequences of nucleotides that code for proteins, which in turn, carry out virtually all the functions of the cell. There are approximately 21,000 genes in the human genome, which makes up only about 2% of the total DNA. The remaining 98% of the genome contains DNA which does not code for proteins but may fulfil a range of other functions, such as indicating the start/end positions of genes and regulating whether or not certain genes are expressed, or "switched on".

4.1.1 Inheritance and linkage

Human cells contain 23 pairs of chromosomes, one inherited from each parent. The process of sexual reproduction involves the fusion of two sex cells (called *gametes*), an egg from the mother and a sperm cell from the father. Each gamete only contains half the full complement of DNA (23 chromosomes). When fertilisation occurs, the genetic material from both gametes combines and the newly created cell obtains its full complement of 46 chromosomes. In this way, an individual inherits 50% of their genetic material from each parent. Meiosis - a random shuffling of genetic material - is a key phase in the process of forming gametes from diploid cells, meaning that no egg or sperm cell is identical. This is a key source of genetic variation in organisms that reproduce sexually. This critical stage means that any offspring will inherit many of the same combinations of genes that their parents have but may also have novel combinations that arise due to this recombination. In this way, a person may share many similarities with their siblings and parents, but may also be quite different.

When the recombination outlined above occurs, the likely inheritance of each gene is not a statistically independent event. The chances of genes being inherited together or being separated during this random shuffling are influenced by physical proximity to each other on the chromosome. Offspring possess two copies (known as *alleles*)

of each gene, one from each parent. Genes that are located at adjacent loci have a higher likelihood of being co-inherited, which leads to the formation of associations between alleles in a population. This is known as *linkage disequilibrium*, or LD (Ardlie et al., 2002). This notion that larger blocks of genes tend to be inherited together has significant implications when designing studies aiming to find genes that cause disease or other genetic traits, and these will be discussed in greater detail below.

In Mendelian terms, alleles can be viewed as dominant, recessive or co-dominant. This quality effects how genetic traits and diseases can be inherited. Diseases that are caused by mutations in single genes follow distinct patterns of inheritance. For example, in order to develop a disease caused by a dominant allele (such as Huntington disease, Walker, 2007), only one copy of the mutated gene is required. It therefore follows that disorders that follow this pattern typically occur in every generation of an affected family. Recessive conditions, where two copies of the mutated allele are required for the disease phenotype to occur (e.g. cystic fibrosis, Bobadilla et al., 2002), often result from individuals inheriting the recessive allele from each parent, who are typically carriers of the recessive allele but who do not have the condition. It follows therefore, that recessive conditions often skip generations. In co-dominant situations, alleles have equal influence on the phenotype that emerges. For example, wavy hair results from the possession of a curly hair allele in combination with a straight hair allele - each allele exerts its influence on the final result and a mixture of the two traits appears. Complex traits, involving the effects of multiple genes, do not necessarily follow these patterns, therefore making the mode of inheritance harder to detect.

4.1.2 Rationale for conducting genetic experiments

In its simplest sense, a genetic experiment seeks to identify a gene which has a causal influence on the phenotype that develops. The phenotype of an organism can be thought of as the characteristics of that organism that develop from interaction between its genotype and environment. The basic hypothesis is that individuals with

a particular trait or disease will have genetic differences to people who do not, and these differences will confer a greater risk of developing the phenotype in question. A genetic study seeks to identify those differences.

The optimal approach depends on numerous factors. Considerable influence over the method of choice is exerted by the nature of the disease under investigation. Is it a Mendelian condition caused by a single gene or a complex condition with effects from multiple loci? The size and make-up of the population of affected individuals available for testing (do you have access to extended families, siblings, unrelated individuals?), and also technical and financial considerations must also be factored in. An in-depth discussion of these factors is beyond the scope of this thesis, but I will briefly discuss both *linkage* approaches (because the existing synaesthesia studies are linkage studies) and *association* methods (because the work conducted for this PhD used this approach).

A study must have a set of identifiable markers which allow the differences between affected and non-affected individuals to be recognised and classified. Genetic markers are sequences of DNA at known locations which can be compared to identify differences between individuals or groups.The current marker of choice used in the majority of studies is the *single nucleotide polymorphism*, or SNP. SNPs are locations in the genome at which individuals differ by a single nucleotide in the string of nucleotides that make up the molecule of DNA. For example, an individual may have a 'G' base at a particular location, when another person may have a 'C'. On a population level, these alleles can occur frequently (e.g., 40% of people may have a 'G' at a certain location and 60% may have a 'C') or they may be rare alleles (e.g., 2% of people may have a 'G' at a given location).

4.1.3 Linkage and association methods

By analysing families with multiple cases, linkage studies aim to isolate areas of the genome that co-segregate with the condition in question, by comparing the pres-

ence/absence of genetic markers between affected and non-affected family members (Ardlie et al., 2002). Linkage approaches work well in detecting the genes linked to conditions that are monogenic (caused by one gene) and highly penetrant and perform poorly when high levels of allelic heterogeneity are present, meaning many alleles at a particular locus have an influence on disease state (Pritchard and Cox, 2002). Linkage studies only tend to identify the loci exerting the strongest influence (Smith and O'Brien, 2005), and candidate regions identified by linkage studies tend to be large (Bailey-Wilson and Wilson, 2011). This means multiple loci exerting smaller amounts of influence are often not detected and the subsequent identification of genes is made difficult by the large size of the search area.

The extent of linkage disequilibrium (i.e. the co-inheritance of adjacent genes) is a significant factor that can influence both major decisions in study design (such as deciding between a linkage or association approach) or more minor decisions (e.g., SNP selection). The closer individuals are related to each each, the higher linkage disequilibrium will be. For example, first degree relatives will have higher linkage disequilibrium than more distant relatives who in turn, have greater linkage disequilibrium than unrelated individuals. This is relevant because higher levels of linkage disequilibrium allow larger areas of the genome to be investigated with fewer markers (Cordell and Clayton, 2005). The advantage of using fewer markers means greater statistical power, and it is technically less challenging and cheaper. The trade-off is a difficulty in identifying the causal genetic variant (i.e. the gene contributing to the cause of the disease) in the often large region of the genome in linkage disequilibrium. Such areas can often contain several hundred genes. The converse applies in situations where linkage disequilibrium is lower. More markers are required to probe the genome, which has implications for statistical power and sample size but the advantage is that areas of association are generally smaller, meaning that causal variants may be easier to isolate.

In practical terms, this means that because first degree family members inherit

much of the same sections of the genome together, isolating the areas of the genome that do not overlap between affected and non-affected members can be achieved with smaller sample sizes than with unrelated individuals, but specifically because those individuals are related, the size of the genomic regions in common are likely to be larger. The implication being that the nature of the the disease under investigation (one gene or many?) dictates the best approach to study it. Conditions thought to be monogenic are more easily identified with linkage studies whereas association approaches are best placed to detect causal influence from multiple genetic variants.

In comparison to linkage studies, association studies work at the population level to determine whether the presence of a particular allele at a polymorphic site correlates with the presence of the condition under investigation (Oksenberg et al., 2008). In other words, when comparing groups of affected individuals with groups of unaffected individuals, is a particular allele more commonly found in that location? Association approaches test for a relationship between the presence of disease and specific genetic variations (Lewis and Knight, 2012). An increased frequency of a particular genetic marker (usually a SNP) in a group of affected individuals implies that that marker increases the likelihood of developing the disease in question. It is important to acknowledge that a positive association is precisely that - just an association. A SNP may fall in the coding region of a gene, in which case that SNP may provide information about the causal mechanism underlying the disease, but more often than not, a SNP may be located in a stretch of DNA that does not code for a gene, or in a gene-poor region of the genome. In these cases, positive associations do not tell us anything about the causal mechanism, but rather act as the drivers of new hypotheses - namely, what genes are in the vicinity of the SNP that may be exerting an effect? Association studies are particularly well suited to detecting common variants that contribute modest risk effects (Risch et al., 1996), which given the current understanding of synaesthesia genetics (see below), makes this approach valid for our own interests. The characteristics of linkage disequilibrium can also be beneficially employed in the

design of association studies. If it is known that certain areas of the genome are in linkage disequilibrium, SNPs can be strategically selected to cover that whole region (Stram, 2004). This ensures greater coverage of the genome for a smaller number of SNPs, effectively increasing statistical power for a sample of a given size.

With regard to selecting SNPs for analysis, there are two broad approaches - a candidate approach or a genome-wide approach. The candidate approach requires an *a priori* hypothesis as to why that specific gene or region is likely to influence the phenotype under investigation. A genome wide association study, or GWAS - is hypothesis free, and examines the whole genome in a predetermined degree of detail, as governed by the number of markers used. One of the primary considerations in a genome-wide association study is the balance between statistical power and sample size. In order to have the required power to detect anything other than the most significant associations, samples of more than 1000 cases (and a greater number of controls) are required. When the study in question aims to investigate a disease that is rare, recruiting such a large sample becomes a significant challenge. For the experimental work presented in this thesis, I adopt the former approach, and select a single SNP that may be linked to synaesthesia (see Chapter 7). The end goal however, is to carry out the first GWAS study in synaesthesia. I am working towards this goal with my collaborators (Julia Simner and Simon Fisher and others), but this falls outwith the remit of this thesis.

A difference between *linkage* and *association* studies is that in association studies, the same allele (or marker) is associated with the trait of interest across the whole population, whereas in linkage studies, different alleles (or areas of the genome) may be associated with the same trait in different families (Cordell and Clayton, 2005). When the genetic origins of a trait are unknown, as is currently the case with synaesthesia, using both approaches is to be recommended. It is possible that the *same* genetic variants give rise to *different* subtypes, or *different* genetic variants give rise to the *same* subtypes. To add further complexity, these possibilities are not mutually exclusive - *both* are also possible. In the following section I will summarise and evaluate the

synaesthesia genetics literature to date.

4.2 The genetics of synaesthesia

Seventy years after synaesthesia was first reported in the scientific literature by Sachs in 1812 (Jewanski et al., 2009), it was observed that this condition was found in multiple members of the same families (Galton, 1883b). This led early researchers to suppose that there was a shared genetic component to this condition which was in some part responsible for the development of the synaesthetic phenotype. This component was identified as being hereditary, enabling the transmission of synaesthesia from generation to generation in some families. Subsequent studies carried out in the modern era place the proportion of synaesthetes that have a first-degree relative who also experiences this phenomenon at approximately 40% (Baron-Cohen et al., 1996; Ward and Simner, 2005; Barnett et al., 2008). Understanding the genetics of synaesthesia offers an opportunity to gain insight into its aetiology and more broadly, may provide a better understanding of how typical human perception develops. It may also help understanding other conditions which might co-occur with synaesthesia, which is the focus of this thesis.

In keeping with the general neglect of synaesthesia by the scientific community in the early to mid 20th century (Lovelace, 2013), the genetic aspect of synaesthesia remained unexplored for most of that time. For the majority of the last century, the techniques required to investigate the potential role of individual genes in synaesthesia were either simply unavailable or too costly. As genetic techniques became available, initial scientific resources were directed towards identifying genes associated with more clinical conditions. Towards the very end of the 20th century however, the first attempts were made to investigate the purported hereditary nature of synaesthesia, and these are reviewed below.

The rebirth of modern synaesthesia research coincided with the development of

suitably reliable methodologies which were able to phenotype the condition, (e.g. test the consistency of the synaesthetic response over time, which is considered to be a hallmark of authenticity, see Chapter 1). At this point, little empirical evidence was available regarding either the prevalence of synaesthesia or the ratio in which it occurs between the sexes. The importance of such information to understanding the inheritance mechanisms of synaesthesia was swiftly realised, and as a consequence, initial studies aimed to establish prevalence and sex ratio and used this evidence as a starting point from which to explore the underlying genetics of this phenomenon (e.g., Baron-Cohen et al., 1996; Simner et al., 2006b).

The first analyses of families with multiple synaesthetes provided evidence to support the idea that synaesthesia may be a penetrant Mendalian disorder with a dominant mode of inheritance (Bailey and Johnson, 1997; Baron-Cohen et al., 1996). As noted above, approximately 40% of synaesthetes report having a first- or second-degree relative with synaesthesia (Baron-Cohen et al., 1996; Ward and Simner, 2005) and there is evidence to suggest this figure may yet be an under-estimation. Because different forms of synaesthesia can co-occur in families (e.g., Ward and Simner, 2003), the presence of a synaesthete with a different manifestation may not be immediately apparent to other synaesthetes in that family. Indeed, systematic screening of first-degree relatives of known probands has revealed that 11% of relatives identified as non-synaesthetes by the proband were actually synaesthetes of a different type (Barnett et al., 2008). Furthermore, when combined with the likelihood that a significant number of people will not be aware that synaesthesia is an unusual phenomenon or will not have discussed it with other family members, the reported recurrence rate of 40% is best interpreted as a minimum figure.

While detailed data for familial recurrence in synaesthesia are limited or non-existent (e.g., recurrence rates for siblings and concordance rates for twins), this initial picture indicates that synaesthesia is a reasonably heritable condition. By means of comparison, the overall lifetime risk of developing multiple sclerosis if you have a first

degree relative with the condition is only 2.9% (Nielsen et al., 2005).

4.2.1 Summary of the current position in the search for synaesthesia genes

At present, the search for genes underpinning synaesthesia is in its infancy. Empirical evidence linking specific areas of the genome to synaesthesia is limited to two published studies[1] (Asher et al., 2009; Tomson et al., 2011), both of which are family based linkage studies. I review these studies below, and evaluate what they tell us about the current understanding of synaesthesia genetics.

Asher et al. (2009) carried out the first whole-genome scan[2] for possible synaesthesia genes using a sample of 196 individuals, consisting of 121 affected and 68 unaffected individuals (7 phenotypes were unknown) from 43 multiplex families. The authors of this study chose a phenotype of synaesthesia they called "auditory-visual" synaesthesia, and which was defined as the experience of colour triggered by sounds and/or spoken words. Today, this would be recognised as two or three distinct variants: grapheme-colour, day-colour and music-colour synaesthesias. After initial analysis using microsatellite markers (short, repeating sequences of base pairs), fifteen potential regions of interest were identified as being potentially linked to synaesthesia. After a secondary analysis carried out at higher density using additional markers, this number was subsequently reduced to four candidate regions. Significant linkage to chromosome 2q24 was found, and in addition, suggestive linkage to chromosomes 5q33, 6p12 and 12p12 was also reported. Because the mode of inheritance is unknown and because preliminary analysis revealed that parametric analysis was a poor fit to the data, Asher et al. (2009) employed a non-parametric approach to analysis. In this context,

[1] An additional study by Gregersen et al. (2013) has been published exploring genetic links between synaesthesia and absolute pitch, but this study used the same genetic data collected for Asher et al. (2009)

[2] A linkage study that covers the whole genome is not the same as a genome wide association study (GWAS). Linkage studies generally require the pedigrees of each participant to be known, so are usually carried out with family groups. Association studies do not require the pedigrees of the subjects, so are typically conducted with unrelated individuals

a parametric analysis requires the specification of a genetic model prior to analysis, whereas non-parametric analyses make no assumptions regarding the inheritance pattern of the condition under investigation. This means that non-parametric analyses are more sensitive to linkage detection than parametric techniques, but with the trade-off being a reduction in specificity due to detected linkage peaks being typically larger (Barrett and Teare, 2011).

Tomson et al. (2011) also conducted a linkage study, using a sample of 48 individuals from five multiplex families, in which they genotyped 24 affected and 24 unaffected individuals. They choose a synaesthesia phenotype they defined as "colour sequence synaesthesia" which includes coloured letters, numbers, weekdays and months. This amalgamation of multiple subtypes had been suggested by Novich et al. (2011)'s finding that certain subtypes of synaesthesia cluster together, so that if a person has a type of synaesthesia that has colour as a concurrent (e.g., grapheme-colour synaesthesia) they are more likely that members of the general population to have additional subtypes that also have colour as a concurrent experience (e.g., day-colour synaesthesia) (see below). Carrying out both parametric and non-parametric analyses, Tomson et al. (2011) report linkage to chromosome 16 in two of the five families they tested. Given that all five families were phenotyped as having "colour sequence synaesthesia" but three families showed no linkage to this region, this demonstrates that this locus is not essential for the development of this form of synaesthesia and other loci are most likely also involved. Furthermore, the sample size in this investigation is small, which limits the strength of any conclusion to be drawn from these data.

4.2.2 Evaluation of the current position

Interpreting the findings of Asher et al. (2009) and Tomson et al. (2011) requires the discussion of several potential confounding factors. It is currently unknown whether synaesthesia is itself a single condition with common genetic roots or an umbrella term for a collection of gentically distinct - yet phenotypically similar - conditions.

Evidence that members of the same family can experience quite different forms of synaesthesia point to a common genetic predisposition to develop synaesthesia in some form. On the other hand, the study by Novich et al. (2011) described above, demonstrated that various subtypes of synaesthesias occur in clusters. Subtypes in the same cluster are statistically more likely to co-occur in an individual, while the probability of experiencing a subtype across clusters remains at chance levels. Novich et al. (2011) identified five independent clusters, which suggests that the development of different types of synaesthesia may arise from distinct neural mechanisms, which may in turn have their own genetic origins.

We have seen here that Asher et al. (2009) and Tomson et al. (2011) report linkage to different regions of the genome. There are several reasons why this may be the case. Each study chose to investigate synaesthetic phenotypes which only overlap in part. Asher et al. (2009) included participants with auditory-visual synaesthesia, defined as participants that experienced colour sensation induced by music and also colour induced by spoken words. Participants in Tomson et al. (2011) experienced a "colour sequence synaesthesia", defined as experiencing colour from either letters, numbers, days or months. It is possible the different regions reflect the differences in phenotype selected by each investigation, and that as per Novich et al. (2011)'s suggestion, each phenotype originates from independent genetic origins. It is unclear how much the phenotypes overlap between studies and indeed how many of the synaesthetes tested may experience multiple forms. Only Tomson et al. (2011) provide a rigorous justification of their phenotype selection, taking Novich et al. (2011)'s findings into consideration. Another difference across studies is that although in both studies, phenotypes were validated by assessing consistency of response, methodological differences make it more difficult to directly compare both investigations. Asher et al. (2009) used a long-term consistency approach (Asher et al., 2006), in which subjects were given a minimum one month interval between tests. In contrast, Tomson et al. (2011) used short-term consistency in which participants were tested with a single test session (us-

ing The Synesthete Battery). For a detailed comparison of single-session and long interval consistency testing, see Chapter 1. Furthermore in Chapter 6, I will also make a detailed empirical comparison of these different testing methods (see Chapter 6).

So in summary, what do these two genetics studies tell us? From the existing evidence, drawing firm conclusions about the genetic origins of synaesthesia is not yet possible. No genes have been discovered and the mode of inheritance has not yet been elucidated. There are several possible reasons why multiple areas of the genome have been identified as being linked to the development of synaesthesia. Independently, both studies emphasise the idea that synaesthesia is most likely a heterogeneous condition. It could therefore be a case of *etiologic heterogeneity*, whereby identical genes result in different phenotypic outcomes, or the opposite, *genetic heterogeneity*, whereby different genes produce the same phenotype. The current evidence does support the idea that synaesthesia is likely to have complex genetic origins, with significant genetic and etiologic heterogeneity likely to be a feature of this phenomenon.

As with many complex genetic conditions, environment-gene interactions may also play a significant role. It is not unreasonable to suggest some form of critical period may exist, whereby exposure to relevant stimuli may influence the synaesthetic phenotype experienced by the individual. To provide a rather extreme example, grapheme-colour synaesthesia is unlikely to develop without exposure to writing in early life. It must also be acknowledged that synaesthesia may be both a "complex trait" and "rare variant" condition. For example, while it is possible that a majority of synaesthesia cases may be caused by the combined influence of numerous genetic loci (i.e., a complex trait), some others may arise as a result of rare or new mutations (i.e., a rare variant). In terms of the phenotype, they may appear identical, despite having distinctly different genetic origins. Although existing evidence may lean towards the former, this does not preclude the possibility that a proportion of synaesthesia cases arise due to rare and novel genetic variants. These unresolved issues in our understanding of the genetics of synaesthesia is one motivation for my own genetics study to be presented

in this thesis (see Chapter 7). Before then however, I address one final issue regarding the genetics of synaesthesia and then move onto an overview of the genes known to be implicated in MS.

4.2.3 X chromosome

A significant early hypothesis regarding the inheritance of synaesthesia concerned the possible involvement of the X chromosome as a region of interest (Bailey and Johnson, 1997; Baron-Cohen et al., 1996). The X and Y chromosomes are the chromosomes which determine sex of an individual; females carry two copies of the X chromosome and males carry one copy of an X and one copy of a Y chromosome. Interest in the X chromosome within synaesthesia research was initially sparked by an apparent lack of father-to-son transmission of synaesthesia, and by early reports in the literature that prevalence that was significantly skewed in favour of females (e.g., Cytowic, 1993). Indeed, early studies reported prevalence ratios as high as 95% female in some cases (Baron-Cohen et al., 1993; see Chapter 1). One possibility to explain this apparent lack of male-to-male transmission and early female bias was that synaesthesia phenotype may be linked to lethality in males in utero (Baron-Cohen et al., 1996). This hypothesis was rejected by Ward and Simner (2005), who observed the ratio of male-female relatives in the families of synaesthetes did not significantly differ from the expected 50:50 ratio.

Given that neither Asher et al. (2009) nor Tomson et al. (2011) report any association with the X chromosome and Asher et al. (2009) present hitherto unreported cases of father-to-son transmission in their sample, it seems unlikely that the X chromosome plays a major role in the genetics of synaesthesia. Recent studies report a more equal sex ratio and provide evidence that previously reported skewed prevalence findings are likely to be due in part to self-referral confounds. Work conducted in this thesis also supports this finding (please see Chapter 7 for a full discussion of my study investigating synaesthesia genetics).

4.3 Genetics of multiple sclerosis

In comparison to synaesthesia, research into the genetics of multiple sclerosis is a well established field stretching back several decades. Hundreds of studies have been carried out, culminating in state of the art genome wide association studies involving more than 10,000 affected individuals (e.g., Consortium et al., 2011, Consortium et al., 2013). Our current understanding of MS points to a complex and heterogeneous condition, the susceptibility to which is governed by a complicated interaction between genes and multiple environmental factors such as nutrition and exposure to sunlight (McElroy and Oksenberg, 2008). This interaction is neatly illustrated by the difference in concordance rates in monozygotic twins of 25-30% and dizygotic twins of 2-5% (Sadovnick et al., 1993), indicating that the genetic component of MS is strong and environmental and epigenetic factors are also significant.

Excluding the influence of the environment on MS, the genetics of this disease are themselves exceptionally complicated. Increased sample sizes, increased ability to sequence the genome in greater detail, lower costs and greater technical capacity for data analysis have led to an increasing number of causative loci being identified. Furthermore, as the field matures, the increasing requirement for findings to be replicated in independent studies before being accepted as bona fide has lead to greater corroboration of existing and novel results. Loci that have a causative effect on the development and aetiology of MS can be broadly divided into two areas, the *Major Histocompatibility Complex (MHC)* (discussed in more detail below) and the rest of the genome. First reported in 1972 (Bertrams and Kuwert, 1972; Naito et al., 1972), the first, most replicated and most significant result in MS genetics was the discovery of the involvement of the MHC locus (McElroy and Oksenberg, 2008). The effect this region on chromosome 6p21 has on MS considerably dwarfs the contribution of the rest of the genome (Consortium et al., 2005;Ramagopalan et al., 2009). Nonetheless, there are many other regions which confer additional risk of developing MS or influence disease outcome in some capacity. To date, 110 causal variants in 108 separate

loci have been identified (Consortium et al., 2013), with more likely to be found in the foreseeable future. The current picture of MS genetics shows a condition characterised by etiologic and genetic heterogeneity, with susceptibility and disease course governed by a complex set of gene-gene and gene-environment interactions (McElroy and Oksenberg, 2008).

4.3.1 The major histocompatibility complex

What is the major histocompatibility complex and why is it of interest in synaesthesia research? The major histocompatibility complex (MHC) - also referred to as the human leukocyte antigen (HLA) in *homo sapiens* - is a 3.5MB region located on chromosome 6p21 (Muers, 2011). It is the most gene dense region of the human genome and is heavily implicated in the immune system and autoimmune disease susceptibility, with an estimated 22% of genes in this region serving an immunoregulatory function (Fernando et al., 2008). One of the primary roles carried out by MHC proteins involves the presentation of antigens to T-cells, allowing the immune system to differentiate between self and non-self cells and initiate an appropriate immune response (Janeway et al., 2001). There are several characteristics of the MHC that make differentiating causative effects of individual loci a significant challenge (Traherne et al., 2006). It is a highly polymorphic region, meaning the genes in this region may exist in many common versions. If a gene linked to a disease or trait exists in many alleles, identifying the specific allele responsible for that disease is more difficult than if the gene existed only in 2 or 3 variants (Trowsdale and Knight, 2013). It is also a gene dense region with high linkage disequilibrium, meaning actual causal genes are harder to differentiate from the many genes close by that are likely to be inherited in concert with the causal gene as a consequence of their proximity.

Given its functional focus on immunity, it is unsurprising that robust associations between loci found at the MHC and a wide range of (auto)immune conditions have been discovered, including irritable bowel syndrome (IBS), Celiac disease, Myasthenia

gravis and Crohn's disease to name but a few (de Bakker et al., 2006). Furthermore, as we noted above, the oldest, most significant and most replicated genetic finding for multiple sclerosis is also found within the MHC (Patsopoulos et al., 2013). As noted, this region of the genome provides the most significant association with risk of developing multiple sclerosis (Patsopoulos et al., 2013), accounting for between 17-62% of genetic susceptibility to MS (Haines et al., 1998). Within this locus, the gene HLA-DRB1 has been repeatedly shown to have the strongest effect on multiple sclerosis. Consequentially, if there is a genetic link between multiple sclerosis and synaesthesia, examining the MHC is one of the most logical places to start looking. In this thesis, I examine whether this specific area of the MHC locus, already strongly implicated in MS, is also linked to the presence of grapheme-colour synaesthesia (see Chapter 7).

4.4 Genetic overlap between multiple sclerosis and synaesthesia

In Chapter 3, I show that the radiological profile of MS is over-represented in synaesthetes participating in MRI studies of synaesthesia. What is the nature of this association? If there is a causal link between these two conditions, investigating the possibility of shared genetic aetiology is a highly worthwhile approach. From a methodological perspective, the vastly more mature MS genetics literature offers a robust and well researched starting point from which to identify areas of the genome that may be implicated in the development of synaesthesia. Furthermore, some of the areas of the genome reported by the studies of synaesthesia genetics discussed above also feature in the MS literature. Specifically, an area of chromosome 2 (2q24) was reported by Asher et al. (2009) and also Jakkula et al. (2010). Suggestive linkage to region 5q33 in synaesthesia was reported by Asher et al. (2009) and in MS by Consortium et al. (2005). Similarly, region 12p12 has been linked to both synaesthesia (Asher et al., 2009) and MS (Vitale et al., 2002). While this is encouraging, caution is also ad-

vised. The maturity and scale of the MS genetics literature means that more than 100 locations in the genome have been reported as being linked to MS and often, many of the effects are small. The regions of the genome listed here are also often quite large, containing many genes. It is therefore possible that even if the same regions of the chromosome are linked to synaesthesia and MS, different genes within that region may be linked to each condition.

4.5 Chapter conclusions

Synaesthesia genetics is a field in its infancy. Existing evidence is limited and points to a heterogeneous condition influenced by multiple loci. MS genetics is significantly more advanced, confirming MS is an autoimmune condition, the majority of the genetic association coming from a large and complex locus on chromosome 6, called the major histocompatibility complex, which plays a fundamental role in immunity.

Given the strong association between the MHC and MS, as well as the compelling evidence that MS is an autoimmune condition, I will take the following approach in the chapters that follow. In Chapter 5, I will present a new model of synaesthesia in which I consider whether it might be best considered under the rubric of autoimmune conditions. In this immune hypothesis of synaesthesia, I discuss a theoretical framework that might link the neurology and genetics of synaesthesia with the finding in Chapter 3 that MS was overly represented in synaesthetes.

Chapter 5

The immune hypothesis of synaesthesia

5.1 Introduction

In this chapter, I introduce the *immune hypothesis* of synaesthesia, a novel hypothesis which proposes the idea that genes which have a dual function - influencing connectivity in development and immune system function in adulthood - may play a causal role in the development of the type of altered cortical connectivity thought to be present in synaesthetes. Sections of this chapter have been published in the form of a journal article, as Carmichael and Simner (2013) (see Appendix E).

We saw above (in Chapter 1) that current explanations of synaesthesia posit structural and/or functional differences in the synaesthete brain, and frame their models in terms of excess cortical connectivity or altered cortical feedback. Yet, it is unclear how this altered connectivity may arise and which mechanisms might regulate it in early development. In this chapter, I propose an immune hypothesis of synaesthesia, which supplements these existing models of synaesthesia by suggesting how such altered connectivity may arise from a genetic standpoint. My hypothesis also provides a theoretical framework by which to further understand possible associations

between synaesthesia and comorbidities linked to immunity. For example, in Chapter 3, I present data showing an unexpectedly high prevalence of MS/RIS in synaesthetes. Because MS is strongly mediated by the immune system (Trapp and Nave, 2008; Consortium et al., 2011), it is plausible that any association between synaesthesia and MS may originate from immune system impairments that are related. In this chapter, I first review the dominant neurological models of synaesthesia introduced in Chapter 1. Next, I review the possible genetic bases of these neurological architectures in synaesthesia. Finally, I show that the genes likely to be implicated in these types of neurological architectures also have an additional function in the mature brain: one of immunity.

In Chapter 1, I described the two categories of model that seek to explain the generation of synaesthetic experiences. *The cross-activation model* (Ramachandran and Hubbard, 2001) suggests that excess connectivity between functional areas of the cortex allows activation in one cortical area (e.g., auditory cortex) to directly trigger activation in another (e.g., visual cortex). Evidence in support of this model comes for example from diffusion tensor imaging (DTI) and shows that excessive connectivity is indeed a feature of the synaesthetic brain (Rouw and Scholte, 2007). *Re-entrant and disinhibited feedback* models propose that synaesthetic sensations are caused by disinhibited feedback from higher cortical areas (e.g., in parietal lobe) failing to suppress non-relevant activation from lower cortical areas (Grossenbacher and Lovelace, 2001). This type of disinhibited feedback may result from excessive activity of excitatory neurons within the delicate balance between both excitatory and inhibitory neurons in the brain (Hubbard et al., 2011). Despite appearing superficially different, connectivity and feedback models need not be mutually exclusive. It is unlikely that altered feedback happens entirely in the absence of changes in cortical connectivity, given the Hebbian principle that simultaneous activity strengthens interconnectivity between neurons. Therefore, these two approaches might be considered somewhat unified in that connectivity models propose aberrant connectivity as the primary causal mecha-

nism underlying synaesthesia whereas feedback models might allow altered connectivity as an indirect consequence of disinhibited feedback.

While these models are now more than a decade old, explanations of how these cortical characteristics might arise have proven elusive thus far (but see Brang and Ramachandran, 2008; Mitchell, 2013) and I explore this here. Synaesthesia is thought to be primarily neurodevelopmental in nature (Spector and Maurer, 2009). Consequentially, known processes of brain development are likely to be implicated in its emergence. I propose that insight might be gained from examining the functionality of genes that regulate the types of altered synaesthetic cortical connectivity assumed in these models above (i.e., genes for axon guidance, synapse density). This is the approach I follow below.

5.2 Dual gene functionality: connectivity and immunity

As noted above in Chapter 1, current models link synaesthesia to altered structural connectivity, misregulated feedback mechanisms, or a combination thereof. Explaining how synaesthesia develops might therefore come from considering the genes known to be responsible for the development of cortical connectivity. The key point I make in the current chapter is that such genes - responsible for processes such as synaptic pruning and axon guidance in early brain development - also tend to have a dual function. Crucially, this function is an immune one (for review, see Boulanger, 2009). I therefore ask here whether a propensity to develop synaesthesia via atypical regulation of synaptic pruning may be linked to the expression of genes also implicated in immunity in the CNS, since this expression can be conferred by genes with functions of both immunity and cortical development. Put differently, I ask whether synaesthesia may be linked not only to altered cortical connectivity but also to the expression of immune genes. Many genes have been shown to have precisely the dual functionality

described above (Boulanger, 2009). As well as their immunity function, such genes act in cortical development, altering structural and/or functional connectivity by influencing the development of axonal guidance, synaptic connectivity, and synaptic pruning. One outcome of these changes may therefore be the anomalous pattern of connectivity proposed by the cross-activation theory in the development of synaesthesia. Alternatively, the immune system could have a direct influence on excitatory neuronal activity, leading to the outcomes proposed by disinhibited feedback models. This is because the immune system plays an important role in the initial development and subsequent plasticity of glutamatergic synapses, the primary excitatory transmission pathway in the mammalian cortex (Fourgeaud and Boulanger, 2010). Below, I evaluate this novel theory in more detail.

5.3 Can the immune system influence the development of the brain?

Is it plausible to make a link between the immune system and regulation of the central nervous system (CNS)? Isolated from the rest of the body by the blood-brain-barrier, the CNS was once thought to barely interact with the immune system, leading to the long held view that the CNS was "immune privileged" (McAllister and van de Water, 2009). However, research now shows a complex communication between the CNS and immune system, with wide-reaching consequences for brain regulation and development, both in health and disease (Elmer and McAllister, 2012). Immune proteins are known to play a role at many stages in the developmental pathway. They are integral components of phases critical to brain development and plasticity, such as neuronal guidance, synapse development, and synaptic remodeling (Boulanger, 2009). I therefore hypothesize that CNS and immune system interaction may be the biological mechanism which confers the predisposition to develop synaesthesia.

Which aspects of the immune system are known to exhibit the type of dual func-

tionality under discussion in this chapter (i.e., functionality in both immunity and cortical development)? Several areas of this extraordinarily complicated system are worth highlighting. The complement system is one possible candidate, this being a complex cascade of protein interactions involved in immunity which has also been shown to play an important role in tagging of synapses to be eliminated by pruning during development[1] (Stephan et al., 2012). Another candidate relates to cytokines, which are immune proteins that have also been shown to play significant roles in neurogenesis and synaptic plasticity (Bauer et al., 2007). A third candidate relates to major histocompatibility complex (MHC) proteins, described in the previous chapter. MHC proteins are an integral part of the adaptive immune system found on the surface of the majority of nucleated cells and widely expressed in neurons of the CNS (Boulanger, 2004). In addition to fulfilling a crucial function in immune response, MHC class I molecules and related components are thought to be involved in a range of developmental processes, such as activity-dependent plasticity and synaptic refinement (Boulanger, 2009), in other words, processes responsible for the development of cortical connectivity. As noted in Chapter 4, the MHC locus contains several hundred genes, and has also been widely implicated in a range of autoimmune conditions, such as multiple sclerosis (MS), irritable bowel syndrome (IBS), and rheumatoid arthritis (Fernando et al., 2008). While I have highlighted three specific areas of interest above, I also point out that the immune system consists of many hundreds of individual factors and processes. Given this, the specific suggestions of complement proteins, cytokines and the MHC listed are by no means exhaustive. Nevertheless, I consider them to each be plausible candidates for future investigation.

I end this section by asking whether existing studies into the genetics of synaesthesia would support my immunity hypothesis. In other words, have these studies identified areas of the genome containing immune system genes? I noted above that

[1] A blog writer, C Wright, has written blog posts linking synaesthesia to the complement system. She would like it acknowledged that her ideas were written prior to the publication of this thesis and the article by Carmichael and Simner, 2013

research into synaesthesia genetics is in its infancy and as yet, there are insufficient data to draw firm conclusions. No synaesthesia genes have yet been identified and no firm mode of inheritance has yet been elucidated. However, evidence from the two existing studies on the genetics of synaesthesia (Asher et al., 2009; Tomson et al., 2011) have identified several chromosomal regions of interest, and these regions do indeed contain immune function genes. Asher et al. (2009) found significant linkage to chromosome 2q24 and possible linkage to areas on other chromosomes (5q33, 6p12, and 12p12), while Tomson et al. (2011) identified a candidate region on chromosome 16q12.2-23.1. The authors of both studies draw the conclusion that synaesthesia is likely to be a condition influenced by a variety of genes in multiple loci. Nonetheless, the chromosomal regions of interest highlighted in these two investigations do contain immune function genes (e.g., interleukin-17, a cytokine protein found on chromosome 6p12), although there are many other viable candidates that also lie outwith these regions. As such, prior synaesthesia genetics studies may be suggestive of immunity-linked regions also being linked to synaesthesia but the picture they paint is likely to be more complicated.

5.4 The immune hypothesis as a framework for synaesthesia and co-morbidity

An immune hypothesis of synaesthesia might additionally explain recent co-morbidity data which suggests that having synaesthesia may be associated with increased risk of other clinical conditions. In Chapter 2, we saw that Carruthers et al. (2012) report an association between synaesthesia and IBS, having found an elevated prevalence of synaesthesia in a population of IBS patients. Other researchers have raised the possibility that synaesthesia may also be found at elevated rates within populations with autism (Baron-Cohen et al., 2007) or migraine (Alstadhaug and Benjaminsen, 2010). I point out here that the immune system plays a prominent role in all of these conditions (Collins, 2002; Bruno et al., 2007; Enstrom et al., 2009), suggesting that altered immune system function may be a common causal link. If so, my immune model

proposes a plausible framework by which to investigate comorbidity between synaesthesia and other conditions. If this hypothesis is correct, one might ask whether the prevalence of synaesthesia is also higher in populations with other autoimmune conditions. Indeed, the experiment I presented in Chapter 3 suggests that developmental synaesthesia might occur more prevalently in people with MS/RIS, a demyelinating disease of the human CNS, in which a maladaptive immune system is an undisputed factor (Trapp and Nave, 2008), and in which the majority of genes implicated do have an immune function (Gourraud et al., 2012). This statistical link between MS/RIS and synaesthesia, along with a plausible immune hypothesis of synaesthesia, might therefore lead us to investigate whether synaesthesia and autoimmune conditions such as MS could share overlapping genetic origins, and this is the aim of the two chapters that follow.

5.5 Conclusions

In this chapter, I proposed a novel hypothesis that CNS/immune system interactions during early life may play a role in the development of synaesthesia. I have asked whether genes with dual functionality in both brain development and immunity may be at the origin of existing models of synaesthesia, and whether this mechanism could provide a framework to investigate associations between synaesthesia and other immune-related conditions. Identification of genes that contribute to the development of synaesthesia will make a significant contribution to the validity of this hypothesis, and whether synaesthesia has one cause or many. Given this, I present a phenotypic and genotypic analysis of synaesthesia in the following two chapters.

Chapter 6

Phenotyping synaesthesia: Validating the Synesthesia Battery's short-term test for synaesthesia

6.1 Introduction

Previously, in Chapter 5, I presented the immune hypothesis of synaesthesia, outlining a theoretical framework by which synaesthesia, immunity and immune related comorbid conditions (e.g., MS) may be linked by common genetic origins. In this and the following chapter, I introduce empirical work relating to the phenotyping and the genotyping of synaesthesia. Phenotyping relates to how to identify genuine synaesthetes via their behavioural traits, and is an essential step for accurate genotyping of synaesthesia (i.e., looking for areas of the genome linked to synaesthesia).

In order to phenotype (identify genuine synaesthesia), reliable, validated tools are essential. In this chapter, I report empirical work in which I assess what has become the most widely used online test for synaesthesia: The Synesthete Battery (Eagleman et al., 2007). In Chapter 1, we saw that this diagnostic test for synaesthesia relies on assessing the consistency by which synaesthetes report their synaesthetic colours.

Below, I briefly review the nature of this assessment tool and then describe why a validation of this tool would be highly useful before I use it to identify the synaesthetes that will be genotyped in the following chapter. Sections of this current chapter have been published in the form of two journal articles, Simner and Carmichael (2015) and Carmichael et al. (2015) (see Appendices I and G).

Synaesthesia was initially an under-researched and poorly-understood area of cognition until the last decades of the 20th century until the realisation emerged that synaesthetes experiences could be verified behaviourally by the fact that they remain conspicuously stable over time (Jordan, 1917; Baron-Cohen et al., 1987). Specifically, synaesthetes tend to be highly consistent when reporting their synaesthetic sensations for any given stimulus. For example, if the letter J triggers the colour pale blue for a given synaesthete, the synaesthete will tend to repeat that J is pale blue (not green, not yellow) when repeatedly tested over days, months and even years. Indeed, one study was able to show that synaesthetic sensations had remained consistent over at least three decades (Simner and Logie, 2008).

As discussed in Chapter 1, this stability of response over time is considered one of the central features of synaesthesia and is routinely verified in almost every publication on the subject (e.g., Asher et al., 2006;Baron-Cohen et al., 1996;Rich et al., 2005; Ward and Simner, 2003; but see Simner and Ludwig, 2012). In other words, while a wide range of behavioural approaches have been employed to phenotype the synaesthetic experience, experimental methodologies aiming to validate synaesthesia have almost exclusively focussed on the feature of consistency. Researchers selecting synaesthete participants for study first verify the genuineness of each case by requiring their synaesthetes to demonstrate high levels of consistency over time compared to non-synaesthete controls (e.g.,Asher et al., 2006; Baron-Cohen et al., 1996; Simner et al., 2006b). Controls are tested on analogous associations (i.e., they invent colours for the 26 letters, say, and then attempt to recall these later) and typically perform significantly worse than synaesthetes.

Although the majority of contemporary studies rely on this test of consistency for genuineness, the particular instantiation of the test has varied widely. For example, a wide range of methods have been used to elicit synaesthetic colours: participants have indicated these by either giving verbal descriptions (e.g., Ward et al., 2005), written descriptions (Simner et al., 2006a), using Pantone© swatch colour charts (Asher et al., 2006), electronic colour charts (Simner et al., 2009a) or even computerised colour pickers offering extensive palettes of $>$16 million colours (e.g., Simner and Ludwig, 2012).

Despite this superficial variability, earlier tests of consistency nonetheless tended to rely on one key shared feature: synaesthetes must outperform controls over fairly lengthy re-test intervals. Consider, for example, the most widely cited large-scale screening for synaesthesia (Simner et al., 2006b) in which a large sample of participants were opportunistically recruited from the communities of Edinburgh and Glasgow Universities, and individually assessed for synaesthesia. Participants first indicated by questionnaire whether they believed they experienced synaesthesia, and those who reported in the affirmative were asked to provide their synaesthetic associations (e.g., the colours of letters). These participants were then retraced after considerable time had passed (on average 6.0 months, but up to several years) and were asked in a surprise retest to re-state their associations. A group of controls without synaesthesia performed an analogous task but were re-tested after only two weeks. Synaesthetes were required to significantly out-perform controls even though much time had passed and the deck was effectively stacked against them. Methodologies such as this allow confident detection of genuine synaesthetes because the surprise retest over lengthy intervals places the performance of synaesthetes beyond the usual abilities of the average person. The drawback to this methodology, however, is that the task is extremely time-intensive to perform, and risks a high drop-out rate if synaesthetes become untraceable at retest.

Perhaps for this reason, one of the most important developments in the method-

ology of synaesthesia validation came with the introduction in 2007 of an alternative version of the test of genuineness. In place of classical long-term retests, Eagleman and colleagues instead championed the short-term retest method. Eagleman et al. (2007) introduced the Synesthesia Battery, a toolbox of online tests which provides a test of consistency measured within a single test session lasting only approximately 10 minutes. Specifically, synaesthetes log on to the testing site (www.synesthete.org) and specify which forms of synaesthesia they experience. The testing platform then presents their triggering stimuli (e.g., the 26 letters) one by one in randomized order, and participants are required to select their synaesthetic colour for each trigger. Each stimulus (e.g., letter) is presented three times each, and a score is generated to quantify the consistency of participant's responses (e.g., did the participant choose the same/similar colours each of the three times she saw a particular letter?) As described in Chapter 1, this score represents the geometric distance in RGB (red, green, blue) colour space, and this score is then normalized to lie between 0 and 1. If the mean overall score of colour-distance is less than 1, the participant is classified as a synaesthete; if the score is 1 or higher, the degree of inconsistency serves to classify the participant as a non-synaesthete. However, it remains an open question whether this limited retest interval is sufficient to truly distinguish synaesthetes from non-synaesthetes.

In this chapter, I assessed the validity of the Synesthesia Battery by using it to test almost 3,000 randomly sampled members of the general population for grapheme-colour synaesthesia. The aim was to establish the prevalence of grapheme-colour synaesthesia by this method. This will allow me to evaluate the Synesthesia Battery by comparing this prevalence - obtained by assessments within a single test session - to the most widely accepted previous estimate of the prevalence of grapheme-colour synaesthesia based on the standard long-term retest method (Simner et al., 2006b). If the Synesthesia Battery is as effective a method for detecting synaesthesia as the more standard long-term retest method, I anticipate an equivalent prevalence of grapheme-colour synaesthesia across both methods. For this study, I chose to evaluate grapheme-

colour synaesthesia in particular for several reasons: it is one of the most common forms of synaesthesia (Simner et al., 2006b), it is particularly well-understood in behavioural terms, it lends itself readily to online testing, and those who experience it typically demonstrate the high levels of consistency expected from synaesthetes (compared to other variants, whose more complex concurrents make them more difficult to assess via consistency alone; see Simner et al., 2011 for discussion). My ultimate aim in this pursuit is to establish whether The Synesthete Battery is an effective way to phenotype grapheme-colour synaesthetes, and to subsequently identify a group of randomly sampled grapheme-colour synaesthetes by this method. These synaesthetes will be genotyped in Chapter 7.

6.1.1 Prevalence and sex ratio

In Chapter 1, I introduced two important questions in synaesthesia research; namely its prevalence and the perceived difference in prevalence between the sexes. In addition to evaluating the Synesthesia Battery, the data generated in this chapter will also provide an independent test of the prevalence of grapheme-colour synaesthesia and explore whether synaesthesia is significantly more prevalent in females. The baseline study to be used as comparison with the data I generate from The Synesthete Battery will be the widely cited prevalence study of Simner et al. (2006b). This study found the prevalence of grapheme-colour synaesthesia to be 1.4% (for synaesthetes with both coloured letters and numbers) or 2% (for synaesthetes with either coloured letters or numbers). This study was based on a sample of 500 individuals, and the prevalence rate it generated was subsequently verified by a secondary method testing a further 1190 individuals (see Discussion for details of this second method). This current study provides an opportunity to replicate these findings with a sample of almost 3000 additional individuals, individually assessed. The individuals in this study were randomly sampled (see Methods for details) and did not rely on self-referral. As such, the current study will provide the largest estimate of prevalence of grapheme-colour synaesthetes to date, and thereby provide a worthy comparison to the previous study I will use as a

baseline.

My investigation will also allow any potential difference in prevalence of synaesthesia between the sexes to be explored. Because self-referral bias during recruitment will be reduced to a minimum in the current study (see Methods) these data will allow me to test the hypothesis that any sex difference found in early studies (e.g., Baron-Cohen et al., 1996) was likely the result of self-referral bias (where women are more likely than men to self-refer). In our baseline study in contrast, Simner et al. (2006b) reported no sex differences, with an overall prevalence of grapheme-colour synaesthesia of 1.1%, with a female prevalence of 1.03% and a male prevalence of 1.15%. In other words, when participants are randomly recruited, the sex difference in synaesthesia is minimised. Using the same random recruitment methods here should therefore also produce no sex difference in the prevalence of synaesthesia. Finally I point out that clearly establishing whether there is or is not a skewed sex ratio in the prevalence of synaesthesia will not only help us confirm whether The Synesthete Battery is a valid way to phenotype synaesthesia, it will also be useful in its own right in my consideration of synaesthesia's genetic aetiology (see Chapter 7).

6.2 Experiment 2

The aim of this study is to assess a large sample of randomly recruited members of the general population for grapheme-colour synaesthesia. This will provide us with a prevalence estimate of grapheme-colour synaesthesia in the general population, which I can then compare to the most widely accepted prevalence estimate in the literature (Simner et al., 2006b). If these prevalence estimates match, we can conclude that the short-term consistency testing of The Synesthete Battery is as an effective method for phenotyping synaesthesia as classical long-term methods.

6.2.1 Methods

6.2.1.1 Participants

Two thousand eight hundred and forty-seven participants took part in this study (1317 male, 1530 female; mean age 28.6, range 16-90, SD 14.3). Thirty-two further subjects completed our study but had entered an obviously false date of birth (e.g., 2013). These subjects did not enter our analysis, which was therefore based only on our N=2847.

Participants were recruited as part of a large-scale, centrally co-ordinated undergraduate research project. Every student registered on the 2nd year of the Psychology undergraduate course at the University of Edinburgh acted as a research assistant (RA), and was required to each recruit 8 participants (4 male and 4 female) over 16 years of age. The student RAs were not allowed to take part in the study themselves. In recruiting the participants, I took a number of steps to ensure as random a sample as possible. First, RAs were instructed not to deliberately seek out, nor to avoid, people they knew to be synaesthetes. Furthermore, in order to avoid self-referral biases, RAs were required to pre-select their sample, and then approach participants in a targeted way (rather than send out an advert and accept self-referrals). Indeed, RAs were required to refrain from recruiting participants via any open calls at all, for example, they could not post the testing URL on social media websites or internet forums. Finally, RAs were also instructed not to a priori inform participants that the study involved synaesthesia.

The study was carried out in two waves to maximise participation numbers: 1514 were tested in January 2013, and 1333 were tested in September 2013. Both used identical methods, carried out by two consecutive intakes of 2nd year students. Data from both rounds are pooled and presented together here.

6.2.1.2 Materials/Procedure

My test was presented online and participants were sent an email directing them to the url. The online test consisted of several sections. Participants first provided informed consent via a checkbox and then gave demographic information such as age, sex, handedness and native language. A second section consisted of a health questionnaire in which subjects were requested to indicate if they suffered from a range of clinical conditions. These data were used to examine comorbidity in synaesthesia and are reported in Chapter 9). After this page, the short-term consistency test of synaesthesia began. In this, we created a locally stored replica of the Synesthesia Battery, and so all the methods hereafter pertain to this exact replica of that consistency test. Participants were first asked whether they experienced grapheme-colour synaesthesia, with the question "Do numbers or letters cause you to have a colour experience?", This was accompanied by an example, and then an option to accept separately according to whether these colours are triggered automatically by numbers and/or digits. If participants indicated that they saw neither letters nor numbers in colour, they advanced to an exit page thanking them for their participation. However, those who answered in the affirmative went on to complete the short-term consistency test, cloned identically from the Synesthete Battery. Note that the consistency test was presented only to those who first self-declared having synaesthesia, and this is the established method for identifying synaesthetes (e.g., Simner et al., 2006b).

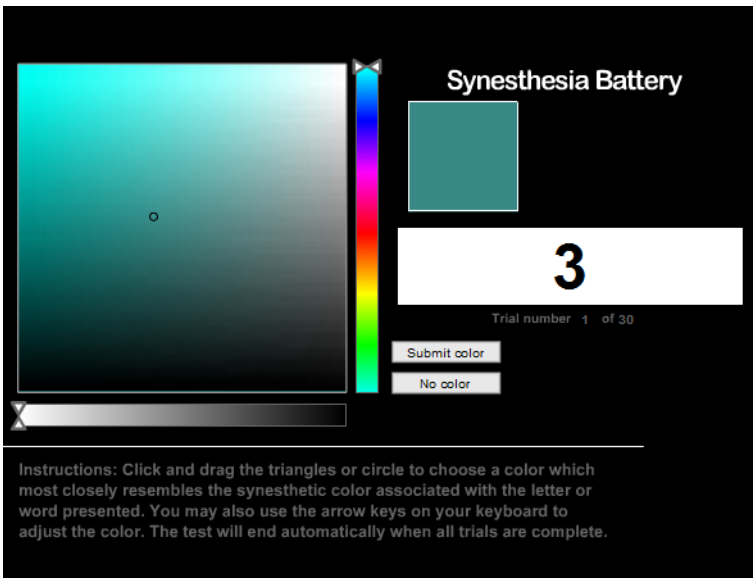


Figure 6.1: **Screenshot from the consistency test**

Participants who completed the short-term consistency test did so only for their self-declared particular variant of grapheme-colour synaesthesia (for either digits, letters, or both). This consisted of two parts, a colour consistency test and a speeded-congruency task (see below). The colour consistency test was an identical clone of the consistency test from the Synesthesia Battery (Eagleman et al., 2007). In this, participants were presented with each grapheme three times in random order (so 30 trials if the subject reported coloured numbers only, 78 trials if letters only, and 108 trials if both letters and numbers). For each trial, participants were required to select the colour that "best matched" the grapheme presented (see Figure 5.1). Selections were made from a palette of 256x256x256 colours. Once their selection was submitted, the screen advanced to show the next grapheme.

The colour consistency test was followed by a speeded-congruency task. This speeded congruency test is also a feature of the Synesthete Battery but it not used as the primary diagnostic measure of synaesthesia. For this reason, as well as a programming issue encountered here, this test will not be considered further.[1]

[1] After the current single session short-term test of consistency of coloured graphemes, participants next immediately perform a second test of synaesthesia: a speeded-congruency verification task (Eagleman et al., 2007). In this section of the Synesthete Battery, participants are shown again the graphemes

6.2.2 Results

The short-term consistency test generated a consistency colour-distance score. Here, I follow the threshold implemented in Eagleman et al. (2007), meaning any participant scoring < 1 on the short-term consistency test is considered to be a genuine synaesthete. For full details of website configuration and how the consistency colour-distance score is calculated, see Eagleman et al. (2007). In my analysis below, I classified as non-synaesthetes all those who were directed to the early-exit page (i.e., those who said they did not experience coloured letters and/or digits) and all those who continued but scored 1 or higher. The remainder were classified as synaesthetes (i.e., those who scored < 1).

6.2.2.1 Synaesthesia prevalence

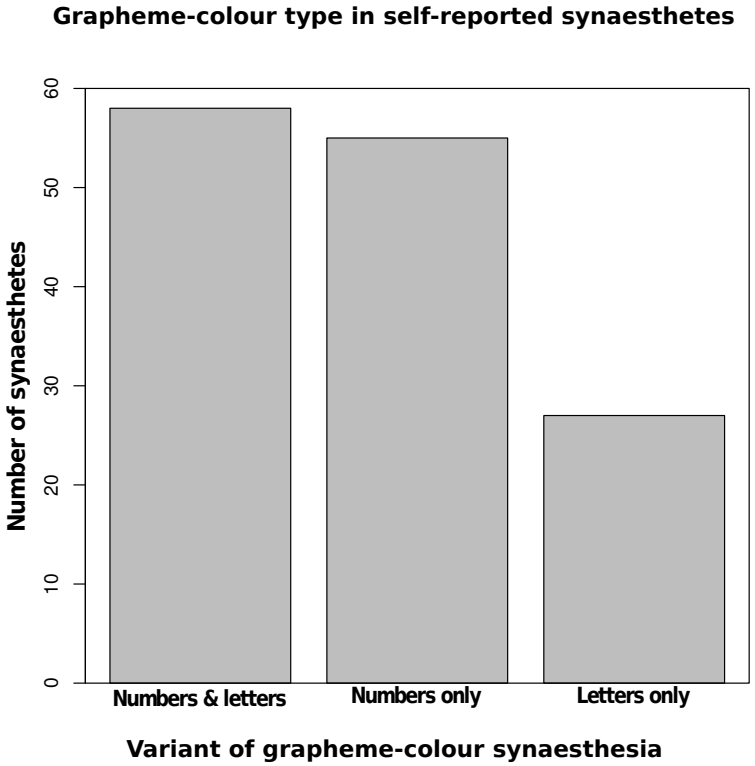
From this sample of 2847 participants, 140 subjects (55 male, 85 female, mean age 23.9, range 16-71, SD 9.5) self-reported grapheme-colour synaesthesia, giving a self-reported prevalence of 4.9%. Of those 140 self-reported synaesthetes, 34 obtained a

they had just seen in the colour consistency test. This time they see each relevant grapheme twice, in a random order, each flashed on screen for a maximum of 1 second or until the participant responds (20 trials for just numbers, 52 trials for just letters or 72 trials for both letters and numbers). In 50% of trials, graphemes are coloured congruently with the participant's earlier specification, and in 50% of trials they are coloured incongruently. Participants are required to indicate by mouse-click whether each grapheme they saw either matched or didn't match their previous colour pairing (as collected during the consistency test; Figure 5.2). Their response mouse-click advanced the test to the next grapheme, and the test continued until all graphemes had been shown. On completing this study we discovered we had programmed minor methodological differences to Eagleman et al. (2007) in how congruent and incongruently coloured graphemes were selected and presented. In this study , the first presentation of graphemes included 50% that were randomly selected to be presented in their congruent colour, taken from the participant's first selection in the prior task. For the second presentation, 50% of graphemes were again randomly selected to be congruently coloured, this time from the participant's second selection from the previous task. The incongruent colours were selected to be sufficiently distant in RGB space from the congruent colours to prevent the two being confused. However, these incongruent colours were randomly selected in RGB space, and were not taken from the participant's earlier palette. In Eagleman et al. (2007), each grapheme was presented twice in a fully randomised order once congruently (always from the 1st selection given previously) and once incongruently. The incongruent colour was chosen from the participant's earlier palette of colours on the consistency test and verified to be sufficiently distant in RGB space from the congruent colour. The details of this were not given in the Eagleman paper.) Furthermore in this study, a response press advanced the display onto the next grapheme, while in Eagleman et al. (2007) the grapheme was displayed for a constant 1s followed by a fixed inter-stimulus interval. These differences mean I can no longer directly compare my data in this section to those from Eagleman et al. (2007). Finally I point out that our central aim to assess the consistency portion of the Synesthesia Battery is unaffected, because this omitted section is not widely used for this purpose in any case.

colour-distance score of < 1 on their consistency test (14 male, 20 female, mean age 24.9, range 17-51, SD 5.8), which is the criterion used by Eagleman et al. (2007) to identify genuine synaesthesia. This places the prevalence of genuine grapheme-colour synaesthesia at 1.2%.

Of the 140 self-reported synaesthetes, 55 reported experiencing coloured numbers only, 58 reported experiencing both coloured numbers and letters and 27 subjects reported experiencing coloured letters only (see Figure 6.2a).

(a)



(b)

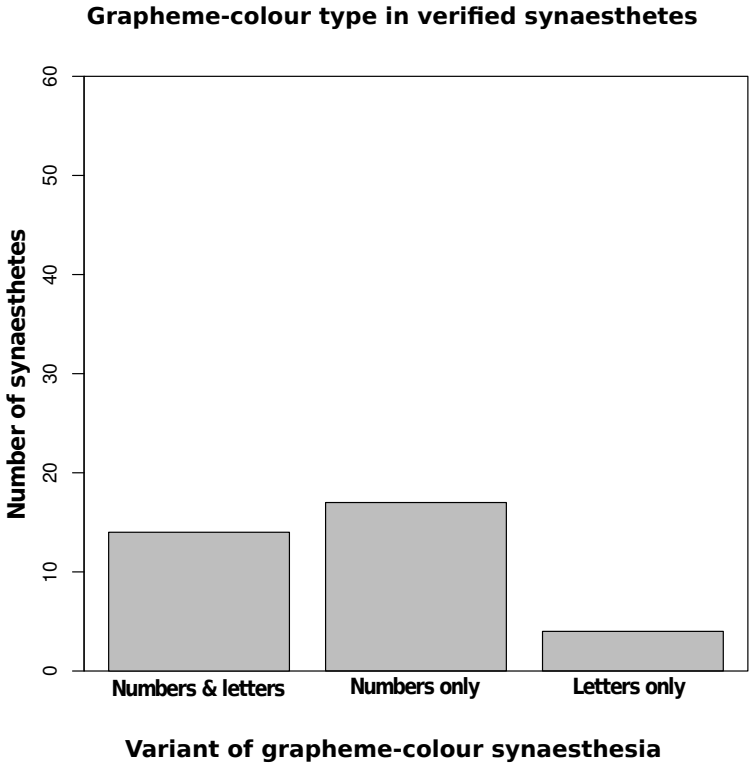


Figure 6.2: **Variants of grapheme-colour synaesthesia reported by (a) all 140 participants who self-reported synaesthesia, and (b) the 34 participants confirmed as genuine synaesthetes (i.e., with colour-distance consistency scores of** < 1**).**

Of the 34 participants that scored < 1 on the consistency test, 17 experienced coloured numbers only, 14 experienced both coloured numbers and letters and 3 subjects experienced coloured letters only (see Figure 5.3b). As an additional check, the colour choices of the participants obtaining a consistency score of < 1 were examined individually to allow us to confirm that none of these achieved their superior consistency by entering the same colour for each grapheme, or by entering an obviously non-synaesthetic pattern of colours throughout, e.g. red for R, green for G and blue for B (see Simner et al., 2006b).

6.2.2.2 Comparing short and long-term consistency testing

The key aim of this study was to compare the prevalence of grapheme-colour synaesthesia generated by the Synesthesia Battery (a short-term, single-session test) to a more conventional method based on long term (rather than single session) testing (Simner et al., 2006b). Using the Synesthesia Battery, the prevalence of grapheme-colour synaesthesia in the general population was found to be 1.2% for those with coloured letters or digits, compared to the previous estimate of 2% in conventional longer-term testing (Simner et al., 2006b). These two estimates are not significantly different (χ^2 = 2.1; df = 1; p = 0.14). However, if the prevalence for synaesthetes with both coloured letters and digits is calculated, a value of 0.5% is found, which is significantly less than the 1.4% found in conventional longer term retesting (Simner et al., 2006b; χ^2 = 5.6; df = 1; p = 0.02).

One recent study has also speculated that the threshold in the Synesthesia Battery perhaps should be raised. Rothen et al. (2013) have recently argued that a more appropriate cut-off may indeed be higher, and their argument relates to considerations of colour space. Eagleman et al. (2007) evaluates consistency using distance in RGB space but Rothen et al. (2013) suggest that alternative colour models (CIELUV and CIELAB) might provide more sensitive measures. On this basis they proposed a revised threshold within the existing Synesthesia Battery at < 1.43 for synaesthetes

(rather than < 1). In this study, if prevalence is recalculated in this sample using Rothen et al. (2013) suggested cut-off score of 1.43, our prevalence estimates are no longer significantly lower than expected. With this revised threshold, I now find 70 genuine synaesthetes with coloured letters OR digits (2.5%; compared to 2% in Simner et al., 2006b; χ^2 with Yates correction = 0.4; *df* =1; $p = 0.53$) and 31 synaesthetes with coloured letters AND digits (1.1%; compared to 1.4% in Simner et al., 2006b; χ^2 with Yates correction = 0.143; *df* =1; $p = 0.706$). In other words, our results are more in line with more conventional longer term evaluations of synaesthesia when the threshold is shifted upwards according to the proposal of Rothen et al. (2013).

6.2.2.3 Sex ratio

In addition to using total prevalence as a proxy for evaluating the validity of the Synesthete Battery, comparing the prevalence of female and male synaesthetes is also a useful additional benchmark. In this investigation, a total of 2847 participants were tested, 1530 female and 1317 male. From this sample, we identified a total of 34 grapheme-colour synaesthetes, giving an overall prevalence of 1.2%. Of these 34 synaesthetes, 20 were female and 14 were male. Examining the synaesthesia prevalence of each sex separately gives us a female prevalence of 1.3% and a male prevalence of 1.1%. The difference in prevalence between males and females is not significant ($\chi^2 = 0.358$; *df* = 1; $p = 0.55$).

These results corroborate the findings of our baseline comparison study. In the museum part of their study (testing 1190 visitors to a science museum), Simner et al. (2006b) reported an overall prevalence of grapheme-colour synaesthesia of 1.1%, with a female prevalence of 1.03% and a male prevalence of 1.15%. Comparing the sex ratio results from my current study with the results of Simner et al. (2006b) using a Cochran-Mantel-Haenszel test reveals no significant difference ($\chi^2_{MH} = 2.14$; *df* = 1; $p = 0.14$). In other words, the difference in sex ratio in this investigation does not differ significantly from the ratio found by Simner et al. (2006b).

Examining these data acquired in my study from a Bayesian perspective corroborates the above statistical interpretation. By comparing the likelihood of two models (in this case, the null and alternative hypotheses) as a ratio, Bayes factors allow us to evaluate to what extent the data supports the hypothesis under investigation (Rouder et al., 2009). Following Jeffreys (1961), a Bayes factor of less than 0.33 provides strong support for the null hypothesis, a Bayes factor of greater than 3 provides support for the alternative hypothesis and values in between indicate the data are insensitive and no firm conclusions should be drawn. We calculated a Bayes factor of 0.012, indicating that the sex of the person does not significantly alter the likelihood of having grapheme-colour synaesthesia.

6.2.3 Discussion

In this study, I reproduced elements of the Synesthesia Battery (Eagleman et al., 2007) which is a short-term online consistency test to diagnose (i.e. phenotype) grapheme-colour synaesthesia. One aim of this chapter was to verify this as a valid method to phenotype synaesthetes (in preparation for the subsequent genotyping of these synaesthetes in Chapter 7). I also used this method to estimate the prevalence of grapheme-colour synaesthesia in a very large randomly recruited sample - indeed the largest sample for this purpose to date. I found the prevalence of grapheme-colour synaesthesia in the general population to be 1.2% for those with coloured letters or digits, compared to the previous estimate of 2% (Simner et al., 2006b). These two estimates are not significantly different (χ^2 with Yates correction = 1.55; df = 1; p = 0.2131). However, if the prevalence of synaesthetes with both coloured letters and digits is calculated, a value of 0.5% is found, and this is significantly less than the 1.4% found in conventional longer term retesting (Simner et al., 2006b; χ^2 with Yates correction = 4.3; df = 1; p = 0.04). Hence, the Synesthesia Battery numerically under-estimated the prevalence of those with coloured letters and digits, compared to longer retesting methods. The possible reasons for this are explored below.

One explanation for an under-estimation of prevalence in the Synesthesia Battery might stem from the way graphemes are presented to those who report more than one variant: letters and digits are presented together, randomly ordered within the same consistency test. In longer term retesting however, letters and digits were always presented in separate blocks (Simner et al., 2006b). It may therefore be that synaesthetes are susceptible to interference when selecting colours for graphemes, and I raise this possibility for future studies to consider.

One recent study has also speculated that the threshold in the Synesthesia Battery perhaps should in fact be raised. Rothen et al. (2013) have recently argued that a more appropriate cut-off may indeed be higher, and their argument relates to considerations of colour space. Eagleman et al. (2007) evaluates consistency using distance in RGB space but Rothen et al. (2013) suggest that alternative colour models (CIELUV and CIELAB) might provide more sensitive measures. On this basis they proposed a revised threshold within the existing Synesthesia Battery at < 1.43 for synaesthetes (rather than < 1). The discussion above of where to set the cut-off threshold for consistency in the Synaesthesia Battery might be considered part of a more fundamental concern in synaesthesia research. In all kinds of consistency tests for synaesthesia - and particularly obvious here - participants are divided into two groups by their score on what is in fact an incremental continuum. It seems clear that someone who scores 1.05 on the consistency test is "more synaesthetic" than someone scoring 2.48, yet according to the cut-off of < 1 (or indeed any cut-off), both would be considered non-synaesthetes. Nonetheless, it is a particular strength of the Synesthesia Battery that researchers are free to consider this score in its own right, rather than for categorical groupings alone. It is also worth highlighting that the age range of genuine synaesthetes meeting the cut-off threshold of < 1 is narrower than the age range of self-declared synaesthetes (16-51 years old for genuine synaesthetes, as opposed to 16-71 for the initial self-declared group). In other words, participants over the age of 51 who reported having synaesthesia, were not consistent enough to meet the consis-

tency cut-off of < 1. This is perhaps additional evidence that the threshold should be increased to the < 1.43 proposed by Rothen et al. (2013) in order to increase the sensitivity of the test. Alternatively, this may be evidence that consistency of synaesthetic response does decrease with age, as suggested by Meier et al. (2014).

An additional significant finding from this study is the lack of difference in grapheme-colour synaesthesia prevalence between the sexes. Understanding the source of the difference in numbers of female versus male synaesthetes is important in the context of understanding more about the origins of synaesthesia. As discussed in Chapter 1, earlier findings in the literature suggested that synaesthesia was significantly more common in females than males (e.g., Baron-Cohen et al., 1996). This finding led to the logical conclusion that the development of synaesthesia may be linked to being female in some way. Combining these early findings with evidence showing synaesthesia runs in families and has a genetic component led to speculation that synaesthesia was either not fully expressed in males or may be linked to the X-chromosome, possibly in a way that causes lethality in males *in utero* (on account of having only one copy of the X-chromosome) (Baron-Cohen et al., 1996). However, subsequent research has shown this to be not the case, with families containing synaesthetes equally likely to produce female or male offspring (Ward and Simner, 2005). Furthermore, confirmed cases of male-to-male transmission (Asher et al., 2009) and one case of monozygotic male twins who are discordant for synaesthesia have been reported Smilek et al. (2005). In other words, a higher prevalence of synaesthesia in females might suggest the involvement of the X chromosome in the development of synaesthesia. I do not find this increased female prevalence in this study, therefore these data support later findings that suggest the X chromosome is not involved in the development of synaesthesia.

In this chapter, I have evaluated whether single session tests of consistency are effective at identifying synaesthetes, compared to established longer retesting methods. One previous study has also suggested that single session testing may indeed be valid. Simner et al. (2006b) established the prevalence of synaesthesia both with long term

testing (which I used here as our key comparison) but also by screening an additional 1190 people in a single session. Their method was more basic than that of Eagleman et al. (2007) in that colour choices were made from a palette of just 13 colours, and only absolute matches contributed to consistency scores. Nonetheless, this again produced roughly comparable prevalence estimates as longer term testing (1.1% prevalence for coloured letters and digits). Taken together with the current study, I therefore suggest short-term, single session tests of consistency for synaesthetic associations do appear to provide an appropriate method by which to phenotype synaesthetes. The widely available nature of the Synesthesia Battery through its open-access online interface makes it a particularly appealing version of the consistency test, as does its comprehensive colour palette, and its ability to give a calibrated estimate (i.e., continuous consistency score) for synaesthesia status. Although researchers will want to consider carefully the question of whether consistency captures every type of synaesthesia, or indeed every type of synaesthete (see Simner, 2012 for discussion), it is clear from this current study that the Synesthesia Battery provides a suitable tool for evaluating synaesthetes along this dimension.

Chapter 7

The genetics of synaesthesia

7.1 Introduction

In chapter 3, I investigated a possible association between MS (a condition with strong links to the immune system) and synaesthesia. In chapter 5, I proposed a theoretical link between synaesthesia and genes that play a dual role in cortical development (potentially important for synaesthesia) and the immune system (important for MS). In the previous chapter, I validated the most widely used online assessment of synaesthetic consistency - the Synesthete Battery (Eagleman et al., 2007) - and established the suitability of this tool to phenotype synaesthetes. In this chapter, I present empirical work investigating the potential genetic basis of an association between synaesthesia, MS and immunity. I begin by briefly summarising the rationale for investigating the genetics of synaesthesia and why I limited the phenotype of synaesthesia under investigation to grapheme-colour synaesthesia only. I then discuss the reasons why the specific location of the genome I investigate in this study was chosen. I then explain the phenotyping and genotyping process and present the results of my study. Finally, I discuss the implications of these findings in the context of understanding the genetics of synaesthesia.

7.1.1 Rationale for investigating genetics of synaesthesia

In Chapters 3 and 4, I discussed neurological and genetic reasons why MS and synaesthesia may share common origins, and in Chapter 5, I put forward the hypothesis that genes with the dual function of cortical development and immunity may play a role in the development of synaesthesia. Understanding more about the genetic origins of synaesthesia will help further knowledge of how synaesthesia develops and its neurological basis. Given the increased prevalence of MS-RIS in synaesthetes reported in Chapter 3, investigating whether MS and synaesthesia share genetic origins is of specific interest. There are significant methodological advantages to using MS genetics as a starting point for this investigation. The field of MS genetics is significantly more mature than the synaesthesia genetics literature. Multiple, large scale genome-wide association studies have been conducted, including many thousands of participants (Consortium et al., 2011, 2013). The most significant results from these studies have been replicated, and can therefore be considered reliable. In other words, much is already known about the genetic origins of MS, and exploring genetic commonalities between synaesthesia and MS should begin by taking the most robust associations from this literature as starting points for investigation within synaesthesia.

As discussed in Chapter 4, an apparent high ratio of female synaesthetes and a lack of male-male transmission of synaesthesia led to the hypothesis that the genetic origins of synaesthesia may be found on the X chromosome. Because the observed ratio of male-female relatives in the families of synaesthetes does not significantly differ from the expected 50:50 ratio (Ward and Simner, 2005), and given that neither of the previous studies into synaesthesia genetics (Asher et al., 2009; Tomson et al., 2011) report any association with the X chromosome, empirical evidence in support of this early hypothesis regarding involvement of the X chromosome is limited. For this reason, the X chromosome was not considered to be a potential region of interest in this study. Below, I discuss the rationale behind selecting the region chosen for analysis here.

7.1.2 Selecting the region of the genome for analysis

As first discussed in Chapter 4, SNPs are locations in the genome representing a single nucleotide in the string of nucleotides that make up the molecule of DNA. In other words, at the specific location in the genome denoted by that SNP, there is a single allele; which could either be an 'A', 'G', 'C', or 'T'. SNPs are used as markers in genetic association studies, which is the type of study I will conduct here. The frequency of the particular allele at a given SNP is measured in a sample of individuals who possess the trait under investigation, and this frequency is compared with the frequency of that allele in a control group. If the frequency of the alleles significantly differs, that SNP is said to be associated with the trait under investigation.

First introduced in Chapter 4, the MHC region, located on chromosome 6, is a gene dense area of the genome heavily implicated in both the immune system and autoimmune disease susceptibility. It also contains the most significant and most replicated association with MS reported in the genome, namely allele HLA-DRB1*15:01 (Ramagopalan et al., 2009). Given the strength of its association to MS, investigating whether this allele also has any association to synaesthesia is a logical starting point from which to explore shared genetic links between MS and synaesthesia.

As noted above, genetic association studies (such as this study) use SNPs as markers via which to probe genetic differences at specific locations of the genome. A fundamental question when choosing a SNP to investigate for this study therefore becomes which SNP can be best used as a proxy marker for the allele of interest. A specific feature of the MHC can be used to assist this process of SNP selection. High levels of *linkage disequilibrium* are found in this region of the genome. Linkage disequilibrium (LD) refers to the non-random association of alleles throughout the genome (Slatkin, 2008). In other words, genes that are located at adjacent loci have a higher likelihood of being co-inherited during the process of sexual reproduction, which leads to the formation of associations between alleles in a population. When the likelihood of

alleles being inherited together is high, linkage disequilibrium is said to be high. Because large areas of this region are inherited together (i.e., in linkage disequilibrium), it is possible to identify a selection of SNPs, that are accurate and reliable surrogate markers for larger areas of the MHC region. de Bakker et al. (2006) carried out a comprehensive mapping of SNPs in the MHC, and found certain SNPs can be used as 'tag' SNPs, reliably and accurately acting as proxy markers for larger areas of this area of the genome. Specifically, they found that SNP rs3135388 is almost always in linkage disequilibrium with the allele of interest. Therefore, this SNP represents a highly reliable surrogate marker for the allele under investigation in this study. In other words, if the minor allele frequency at SNP rs3135388 is significantly higher in the synaesthete sample, I can be sure that the frequency of allele HLA-DRB1*15:01 is also higher.

There are three scenarios in which association between a trait of interest and SNP can arise (Cardon and Palmer, 2003). Firstly, a SNP may have a direct causative effect on the trait in question. Second, it may be an indirect effect, arising because the SNP of interest is in linkage disequilibrium with the actual causal variant. Lastly, any association could be spurious, or due to chance (Cardon and Palmer, 2003). Because SNP rs3135388 is almost always in linkage disequilibrium with allele HLA-DRB1*15:01, it is indirect association that would exist in this case (i.e., association to rs3135388 is a surrogate marker for the proposed causal variant, allele HLA-DRB1*15:01).

At the location of the genome chosen for investigation in this study, SNP rs3135388, the possible allele variants are either 'A' or 'G'. In other words, every person either has an 'A' or a 'G' allele at this specific location. The minor allele (i.e., the *least* common allele) is 'A'. Globally, the frequency of the minor allele (the so called *minor allele frequency, or MAF* at this SNP is 0.06. In other words, 6% of people have the 'A' allele at this location. Below, I will discuss how the minor allele frequency varies by geographic location (i.e, by continent continental region) and the implications of this variation for the selection of a suitable control population (see Table 7.1 for the minor allele frequencies in the control population chosen in this study).

In summary, the most significant genetic association to MS is allele HLA-DRB1*15:01, located in the MHC region, on chromosome 6. SNP rs3135388 was selected for analysis in this study because it is the best proxy measure by which to measure the frequency of this allele in the synaesthete sample (de Bakker et al., 2006).

7.1.3 Limiting the phenotype to grapheme-colour synaesthesia

Synaesthesia is a heterogeneous condition, with a wide range of variations having been reported in the scientific literature. In this study, I took a conservative approach to phenotype selection, limiting participant recruitment to grapheme-colour synaesthetes only. Whereas acknowledging this limited my recruitment to a smaller group of available candidates, this decision was taken for the following reasons. Currently, it is not known whether synaesthesia is a single condition or an umbrella term for a range of different conditions that manifest themselves in behaviourally comparable outcomes. It is feasible therefore, that different types of synaesthesia have different genetic origins or developmental mechanisms. If this is the case, recruiting subjects with different synaesthesias could act as a confounding factor in the analysis. There is evidence to suggest that various types of synaesthesia may indeed differ in their development and origin. In a large scale analysis of self-report synaesthetes examining the co-occurrence of different synaesthesia subtypes, Novich et al. (2011) found that different forms of synaesthesia cluster in five separate groups, meaning that a synaesthete with a particular type of synaesthesia was more likely to experience another type in that group but had no greater likelihood of having a subtype from a different group than a control subject. Novich et al. (2011) report that grapheme-colour synaesthesia clusters with day- week- and month-colour synaesthesias, in a group they term “coloured sequence” synaesthesias. This finding suggests that genetic or mechanistic differences may exist between sub-groups and should be considered when conducting genetic analysis.

7.2 Experiment 3

The aim of this experiment is to investigate the potential genetic basis of an association between synaesthesia, MS and the immune system. To do this, a sample of grapheme-colour synaesthetes was recruited, phenotyped to establish the genuineness of their synaesthesia, and then genotyped. The result of this genotyping was then compared to a previously genotyped control population, to ascertain whether the prevalence of the alleles at the SNP location in question was significantly different in the synaesthete sample.

7.2.1 Methods

7.2.1.1 Participant recruitment

Participants for the genetic study were recruited from a range of sources. Grapheme-colour synaesthetes from the Edinburgh-Sussex Database of synaesthete participants were emailed and invited to take part. Online advertisements were placed on student forums and mailing lists at the University of Edinburgh and invitations to participate were included in a variety of media articles. Subjects were also recruited using word-of-mouth and referrals from other researchers in the field. A total of 206 participants were recruited who met the phenotyping criteria outlined below. These grapheme-colour synaesthetes were subsequently genotyped and included in the study.

7.2.1.2 Phenotyping synaesthesia

The first stage in a genetics investigation is the identification of participants who exhibit the trait of interest (also known as the "phenotype"). In Chapter 6, I showed that the Synesthete Battery is a suitable tool with which to identify grapheme-colour synaesthetes, and this methodology was employed here to phenotype participants in the current study. Thus, all prospective participants were required to complete the online synaesthesia assessment battery found at www.synesthete.org (Eagleman et al., 2007). Those who met the required consistency criteria outlined below were included

in the study.

The consistency score of < 1.0, used by Eagleman et al. (2007) as a threshold by which to distinguish synaesthetes from non-synaesthetes, was also used as the primary threshold by which to identify genuine synaesthetes in this investigation. However, an additional consistency rule was also implemented, based on the following empirical findings reported in the synaesthesia literature. Firstly, as discussed in Chapter 6, Rothen et al. (2013) recommend using a more lenient threshold of < 1.43 as a cut-off to distinguish between synaesthetes and non-synaesthetes when using the Synesthete Battery. They conclude that when using the Synesthete Battery, this higher threshold offers greater sensitivity and specificity when distinguishing between synaesthetes and non-synaesthetes. Furthermore, as noted above, Novich et al. (2011) reported that different forms of synaesthesia cluster together. In other words, if a participant were to have grapheme-colour synaesthesia, they would be more likely to also experience another form of synaesthesia from the same cluster (namely day-colour or month-colour forms) but have no increased likelihood (relative to the general population) of experiencing a form from a different cluster (e.g., musical chords-colour).

Therefore, based on the original consistency threshold proposed by Eagleman et al. (2007), and subsequent data from Rothen et al. (2013) and Novich et al. (2011), participants in this study were required to meet a minimum of one of the following consistency criteria:

1. Consistency score of < 1.0 for grapheme-colour synaesthesia (either letters and/or numbers)

2. Consistency score of < 1.43 for grapheme-colour synaesthesia, with a consistency score of < 1.0 for either day-colour or month-colour synaesthesia

7.2.1.3 Genotyping synaesthesia

Once prospective participants had completed the online consistency test for grapheme-colour synaesthesia and met the required inclusion criteria outlined above, a sample of saliva was obtained from each participant. DNA for each participant was then extracted from their saliva sample. Saliva samples were collected using Oragene DNA OG-500 kits (DNA Genotek, Ontario, Canada). To extract the DNA, samples were incubated overnight at 50C and transferred to 15 ml screw-cap tubes. prepIT-L2P reagent (DNA Genotek) was added to the samples (1/25th volume), mixed by vortexing, and incubated on ice for 10 min to precipitate impurities. Samples were centrifuged at 12000 x g in a Sorvall RC6 superspeed centrifuge using a F13-14x50cy rotor with 15 μl tube adaptors. The supernatant containing genomic DNA was transferred to a clean 15 ml tube. Ethanol (1.2 volumes) was added, and the samples were mixed by inversion, and incubated for 10 min at room temperature. The samples were centrifuged for 10 min at 4800 x g in a Thermo Scientific SL40R bench-top centrifuge using a swing-bucket rotor to pellet the DNA. The supernatant was removed and the DNA pellet was washed with 1 μl of 70% ethanol. The DNA was dissolved in 10 mM Tris, pH 8.0, 1 μM EDTA (typically 200-1000 μl, depending on the initial volume of the saliva sample). The DNA concentration was measured using a Nanodrop 8000.

When DNA was extracted from all synaesthete participants, genotyping of the rs3135388 SNP was conducted. In essence, the genotyping process records which alleles are present at the SNP location for each participant. Briefly, this is done by breaking down the DNA structure and washing the DNA over probes that are specifically prepared to be a complementary image of the fragment of DNA at that location. The DNA segment of interest then sticks to this probe and is retained. Technical details of how the process was conducted in this study are as follows. Genotyping of SNP rs3135388 was performed using KASP assay technology (LGC Genomics) and a Bio-Rad CFX96 real-time PCR thermocycler. Each 10 μl reaction contained 0.14 μl of 72X KASP primer mix, 5 l of 2X KASP master mix with standard ROX concentration, and

2 μl of genomic DNA (diluted at 20 ng/μl). Thermocycling was performed as follows: after an initial denaturation of 15 min at 94 °C, 10 cycles were run with 20s denaturation at 94 °C followed by 45s of annealing/elongation starting at 61 °C and decreasing by 0.6 °Cper cycle, followed by 30 cycles with 10s denaturation at 94 °C followed by 45s of annealing/elongation at 55 °C. If necessary, five further cycles were performed using the same parameters as the final 30 cycles from the initial run. The assay was validated by Sanger sequencing of randomly-selected samples of each genotype.

7.2.1.4 Control population

In order to make a meaningful analysis of the genetic information obtained from the synaesthete participants in this study, statistical testing must make a comparison with a suitable control sample. Control data for this study came from the 1000 Genomes Project, a publicly available database of genotyped members of the general public. This database contains data from a globally distributed sample of individuals, and is organised into super-populations, based on geographical origin (African, mixed American, East Asian, European and South Asian). Because the majority of synaesthete participants in this study were European in origin, data from the European super-population were used as control population in the current study. The European population from the 1000 Genomes Project consists of 379 individuals and is further broken down into sub-populations, which are as follows: British in England and Scotland (GBR), Utah Residents with Northern and Western European Ancestry (CEU), Finnish in Finland (FIN), Iberian Population in Spain (IBS) and Toscani in Italia (TSI) (see Table 7.1, below)

Region	Number	Minor allele frequency
GBR	89	0.14
CEU	85	0.15
FIN	93	0.15
TSI	98	0.08
IBS	14	0.11
Total EUR	**379**	**0.13**

Table 7.1: **Genetic control population. The table shows the sub-populations which make up the European super-population, from the 1000 Genomes Project. The numbers of subjects and the minor allele frequency are shown for each sub-group.**

Genetic association studies rely on identifying significant differences in allele frequency at specific locations throughout the genome (i.e., SNPs). The frequency of allele variants at specific SNPs differs depending on the ethnic origin of the person under investigation. In this study, I chose SNP rs3135388 for investigation and above, I reported that the possible allele variants at this location are either 'A' (least common) or 'G' (most common). As shown in Table 7.1, the frequency of each allele variant differs based on geographic origin of the sample under investigation, even within Europe. It is therefore important to match the control sample to the experimental sample as closely as possible, with respect to their geographic region of origin. This prevents differences in allele frequency due to geographical origin confounding the analysis. Because the majority of the participants in this study were recruited in northern Europe, a matching sub-group of controls from this region was used in this analysis.

7.2.2 Results

A range of analyses were carried out, using a selection of models and a variety of control sub-populations. Table 7.2 summarises the analyses conducted in this study. Analyses were conducted using PLINK, the whole genome association analysis toolset (Purcell et al., 2007).

Although it is assumed that synaesthesia has a genetic component, the actual un-

derlying pattern of inheritance is unknown. In other words, it is unknown whether the actual mode of inheritance is, for example, dominant or recessive. The mode of inheritance affects the way in which the underlying genotype manifests itself as the behavioural phenotype. For instance, if the mode of inheritance for synaesthesia was dominant, then a person possessing a minimum of a single copy of the minor allele ('A') at the SNP rs3135388 would exhibit the phenotype of synaesthesia. In other words, a person with 'AA' or 'AG' at this location would be synaesthetic, but a person with two copies of 'G' (the major allele in this case) would not have synaesthesia. If the mode of inheritance was recessive, then the underlying genotype would result in a different prevalence of the phenotype. Only people with two copies of the minor allele (i.e., people with 'AA' at location rs3135388) would exhibit the phenotype of synaesthesia; people with either AG or GG would not be synaesthetic. Because the mode of inheritance is unknown, analyses were run under a number of models, each testing a particular mode of inheritance (see Table 7.2).

As shown in Table 7.2, the four analyses were conducted: Fisher's, genotypic, dominant and recessive, and I explain each test in turn here. Because only four synaesthetes were homozygous for the minor allele (i.e., their genotype was 'AA' at location rs3135388), Fisher's exact tests were used to compare allele frequencies. The Fisher's analysis is a Fisher's exact test without any specific model of inheritance. This test compares the total allele frequencies between cases and controls, irrespective of the specific genotypic combination found in the participants tested. In other words, this test compares the total frequencies of 'A' and 'G' in synaesthetes compared to controls. The remaining three analyses use a Fisher's exact test in combination with a model that tests an assumption relating the underlying genotype found in the cases and controls. Thus, the genotypic test compares the frequency of the three possible combinations of alleles against each other (i.e. AA versus AG versus GG). The dominant model compares the genotypes 'AA' and 'AG' versus 'GG', because under a dominant model of inheritance, the genotypes 'AA' and 'AG' would result in the phenotype of

synaesthesia but 'GG' would not. Lastly, the recessive model tests 'AA' versus 'AG' and 'GG', because under this model, only cases with two copies of the minor allele ('AA') would exhibit the phenotype of synaesthesia.

MODEL	EUR	GBR/CEU	GBR/CEU/FIN
Fisher	0.065	0.423	0.364
Genotypic	**0.020***	0.289	0.083
Dominant	**0.017***	0.257	0.123
Recessive	0.591	0.738	0.287

Table 7.2: **Genetic association analysis. The table shows the results from the genetic association analyses. The type of model and the control population used are shown. * signifies a p-value < 0.05.**

As discussed above, selecting a suitably matched control population is of critical importance when conducting a genetic association study. Here, I conducted the analyses listed above with three different configurations of control population. First, I used the total European super-population, which consists of individuals from British (GBR), northern European (CEU), Finnish (FIN), Iberian (IBR) and Tuscan (TSI) origins. The advantage of using this entire group is the statistical power gained by the larger number of controls (379 in total). The problem with using this group is that the minor allele frequency is considerably lower in the Iberian and Tuscan group (0.11 and 0.08, respectively). As noted above, this difference in frequency can act as a confounding variable. Therefore, analyses were then conducted with control populations that matched the ethic origins of the synaesthete test group more rigorously, controls from British and northern European origin. Because the minor allele frequency is almost the same in the Finnish population, this group was also included, providing an increase in statistical power.

In this study, significant differences in allele frequency were found between synaesthetes and non-synaesthetic controls when the genotypic and dominant models were

tested using the total European control population (see Table 7.2). Because of the potential confound introduced by the difference in allele frequency found in Iberian and Tuscan controls, caution should be used when interpreting this outcome. The interpretation of these results is discussed in greater detail below.

7.2.3 Discussion

The aim of this study was to investigate a potential genetic association between grapheme-colour synaesthesia, MS and the immune system. To do this, I examined a single SNP (rs3135388), which is a surrogate marker for allele HLA-DRB1*1501 (de Bakker et al., 2006). This allele is located in the HLA region of chromosome 6, and has been previously shown to be strongly associated with MS (Benešová et al., 2013; Schmidt et al., 2007; Alcina et al., 2012). Despite strong links to MS, this SNP has not been shown to have associations to other autoimmune conditions. To determine whether this allele is linked to grapheme-colour synaesthesia, the allele frequency at this location in the synaesthete sample was compared with the frequency in a control population.

A range of analyses was carried out in this study, using a variety of models and a range of control population sub-groups. However, when an analysis was carried out using a control population assumed to best fit my synaesthete sample, no significant association between the minor allele frequency and the presence of grapheme-colour synaesthesia was found. Overall, the results of this study were inconclusive. The outcome of the analyses varied considerably, depending on the model tested and the control population used. The most conservative interpretation of these results is that when a control group is used that best fits what we assume to be our test sample, no difference in allele frequency between the synaesthete and control sample was found, and therefore, no association exists between SNP rs3135388 and the presence of grapheme-colour synaesthesia. Because SNP rs3135388 is a very accurate proxy for allele HLA-DRB1*1501 (the strongest association to MS reported thus far in the genome), this study provides no evidence that grapheme-colour synaesthesia and MS

share genetic origins.

There are however, several points that require highlighting to provide a fuller discussion of this null result. Firstly, as noted above, participant recruitment in this study was limited to grapheme-colour synaesthetes only. One of the reasons for this decision was the clustering of synaesthesias reported by Novich et al. (2011). The evidence that different forms of synaesthesia cluster together suggests that the types in each cluster differ in some (as yet) unknown way to types in other clusters. Genetic origins may be one such difference. For this reason, I limited recruitment in this study to grapheme-colour synaesthetes only, to avoid the potential confound introduced by including synaesthesia types with different genetic causes. Should it be the case that different synaesthesia types have different genetic causes, then the possibility exists that while grapheme-colour synaesthesia may not share genetic roots with MS, other forms of synaesthesias might. The null result reported here does not preclude that possibility. Another possibility is that synaesthesia and MS may in fact share a genetic association, but not at the location investigated in this study. Whereas the particular allele in question here is the most strongly associated and most replicated genetic link to MS (Ramagopalan et al., 2009), in total, 110 causal variants in 108 separate loci have been linked to MS to date (Consortium et al., 2013). It is possible that any association between synaesthesia and MS may originate at any of these other loci.

In addition to the above reasons, there is a methodological limitation in how the results of this project can be interpreted. This is related to the available information about the ethnicity of the participants and the subsequent matching of a suitable control population. The purpose of this study is to establish whether SNP rs3135388, (which acts as a surrogate marker for allele HLA-DRB1*1501) is associated with grapheme-colour synaesthesia. To do this, the frequency of the minor allele in the synaesthete population is compared to its frequency in a suitably matched control population. If the frequency of the minor allele is significantly different between populations, this is evidence that the SNP is associated with synaesthesia. The possible confounding factor

is that the frequency of this minor allele varies by ethnicity. For example, in the British population, the minor allele is found in 14% of the population, whereas in the Iberian sample, its frequency is 8%. Unless the ethnicity of the synaesthete sample is known in great detail, accurately matching a suitable control population to the synaesthete sample relies on certain assumptions. It follows that unless the control population and the sample are strictly matched, a proportion of any detected effect could arise due to population stratification differences (i.e., differences in allele frequency as a result of differing ethnicity), rather than due to the presence of synaesthesia. In other words, mismatching test and control populations can result in a confound.

Because detailed ethnicity information is not available for the sample of synaesthetes recruited here, certain assumptions were made regarding the ethnicity of the recruited synaesthete sample. In conducting the analyses above, I am making the assumption that the genetic ethnicity of the synaesthete sample is a sufficiently accurate match with where the participants were recruited (i.e., predominately in the UK and the Netherlands). Making that assumption allows the use of a control sample that matches that geographical location. Unfortunately, these assumptions may not be completely reliable. For example, some participants may have been born and raised in the UK, but have ethnic variation due to immigration in earlier generations (e.g. parents or grandparents). This introduces a degree of uncertainty to the interpretation of the results. Because ethnicity information from the synaesthesia sample is only partially reliable, it is unknown exactly how much population variation exists in this sample, and as a consequence, how closely matched to the control population this sample is. Therefore, some of the difference in minor allele frequency may be caused by differences in ethnicity between the test sample and control group, rather than entirely as a result of the presence/absence of synaesthesia.

The study carried out in this chapter offered the opportunity to investigate potential genetic overlaps between MS, synaesthesia and a region of the immune system. However, the ultimate goal of this work is to conduct the first genome wide associ-

ation study (GWAS) into the genetics of synaesthesia. In genome-wide association studies, the problem described above (population stratification arising from incomplete information regarding the ethnicity of the test sample) is overcome. Because the whole genome is examined, specific markers that reveal the participants' ethnicity can be checked, thus enabling the ethnicity of the entire sample to be reliably established, and rigorously matched to a suitable control population (Pritchard and Rosenberg, 1999). Conducting such a study requires the recruitment of a minimum of one thousand synaesthetes, a scale of recruitment that is outwith the remit of a doctoral thesis. In this chapter, I investigated whether SNP rs3135388, located in an area of the genome with strong links to immunity and associated with MS, was linked to the presence of grapheme-colour synaesthesia. No association was found and I discuss the reasons for this above. In the following chapter, I present empirical data investigating the prevalence of synaesthesia in a sample of MS patients.

Chapter 8

Prevalence of synaesthesia in multiple sclerosis

8.1 Introduction

In chapter 3, I reported the results of an investigation into the prevalence of MS and its clinical precursor, radiologically isolated syndrome (RIS) in synaesthetes volunteering to take part in MRI studies of synaesthesia. In that chapter, I show that the prevalence of MS/RIS is higher in synaesthetes than would be expected in a random sample of the population. In Chapter 7, I conducted a study exploring the genetic basis of synaesthesia to establish whether any links with MS could be found. However, for methodological reasons discussed at the end of that chapter, a conclusive genetics interpretation cannot yet be drawn. Therefore, in order to further investigate a possible association between synaesthesia and MS, I report empirical work in the current chapter that investigates the prevalence of synaesthesia in a sample of multiple sclerosis (MS) patients. Given the findings reported in Chapter 3 in the reverse direction (i.e., an increased prevalence of MS/RIS in synaesthetes), a higher prevalence of synaesthesia in MS patients might also be expected. In the current chapter, three hundred MS patients were recruited from local NHS clinics and all were given a questionnaire that allowed them to self-declare any forms of synaesthesia they believed they had. All

those who indicated they experienced grapheme-colour synaesthesia were given a consistency test for synaesthesia. Those who passed this test were considered as genuine grapheme-colour synaesthetes. Contrary to my hypothesis, the prevalence of synaesthesia in MS patients was not found to be higher than in the general population, and I discuss scientific and methodological explanations for this null result at the end of the chapter.

8.2 Experiment 4

In this study, I evaluate whether the prevalence of synaesthesia is higher in MS patients versus the general population for one synaesthesia subtype - grapheme-colour synaesthesia. There are several reasons why tests were limited to this single subtype. First - and as noted above - it is one of the most common subtypes (Simner et al., 2006b), and it is behaviourally well understood. Second, it is relatively easy to reliably test for and those who experience this form of synaesthesia typically demonstrate relatively high levels of consistency (Simner et al., 2011). Lastly, a suitable baseline prevalence of grapheme-colour synaesthesia was obtained in Chapter 6, allowing direct comparisons of prevalence to be made between MS patients and healthy controls.

In the current study, I recruited a large sample of MS patients who were asked to indicate in a questionnaire whether they had synaesthesia, and to indicate which variants in particular from a list of 162 potential variants (see Methods). After this initial screening, I then sought to verify grapheme-colour synaesthesia using a test of consistency in all those who reported this particular variant. The methods and results are given below.

8.2.1 Methods

8.2.1.1 Participant recruitment

Participants were patients with MS who were opportunistically recruited from specialist neurology clinics run by the Department of Clinical Neuroscience within NHS Lothian. Four clinics per week are run by NHS Lothian and I attended approximately 1-2 per week between October 2012 and August 2013. A total of 71 clinics were attended. Due to stipulations required by the NHS ethics committee, participants were initially approached by a member of their primary health care team; either a consultant or a specialist MS nurse. At this point, prospective participants were asked whether they would be interested in taking part in a study being run in the department. If they expressed a willingness to take part, we were introduced. Subjects were given a written information sheet about the study (see appendix A) and a verbal overview of what the study would entail.

8.2.1.2 Power calculation

The aim of this study was to estimate the prevalence of grapheme-colour synaesthesia in a sample of patients with MS. In order to ascertain the sample size of MS patients required, *a priori* power calculations were carried out to establish how many MS patients should be tested to detect a higher prevalence of synaesthesia in MS, should this exist. To do this, I first had to decide where to set any presumed estimate of synaesthesia prevalence in MS patients. Using the baseline prevalence of grapheme-colour synaesthesia in the general population reported by Simner et al. (2006b) of 2% as a starting point, in order to detect a presumed difference in prevalence within MS patients of - say - 5%, a sample of 307 participants would be required. In other words, if the prevalence of grapheme-colour synaesthesia in multiple sclerosis patients is in the region of 7%, a sample of 307 subjects would have sufficient statistical power (given the convention of 80% power and 0.05 alpha) to reliably detect this difference. Note that the prevalence baseline was taken from Simner et al. (2006b), rather than Chapter

6 of this thesis, since the latter had not been generated at the time of testing. Nonetheless, both studies (Simner et al., 2006b and Chapter 6) produced largely equivalent findings (see Chapter 6).

8.2.1.3 Participants

A total of 300 patients with a diagnosis of MS participated in the study (mean age = 41.1, SD = 11.9, range = 19.5-81.5; 74%female; 9.7% left handed, 1% ambidextrous). A total of 37 additional patients were asked but declined to take part (75.6% female). Reasons for non-participation were predominately a lack of time. A further 58 (69% female) patients who were visiting the clinic for scheduled appointments during my attendance were not approached to take part by the primary health care team. There were a range of reasons behind the decision not to approach patients for participation. Patients deemed too upset were not asked to take part and patients with cognitive or physical impairment that would have obviously prevented the patient from either understanding the nature of the study or completing the questionnaire were also not asked to participate. At the time participant recruitment was completed for this study, 1668 people had been diagnosed with multiple sclerosis in the Lothian region (1201, 72% of whom were female).

8.2.1.4 Initial screening

Once the patient agreed to take part, he/she completed an initial screening stage of the study during their appointment at the clinic. Initial screening consisted of the completion of a consent form (see appendix B) and the administration of a screening questionnaire (see appendix C), which aimed to identify participants who felt they experience some form of synaesthesia. The questionnaire was an adapted version of the questionnaire used by Simner et al. (2006b), and it explained what synaesthesia is, provided examples of synaesthesia and then asked subjects to indicate which forms of synaesthesia they experienced, if any. They did this by drawing lines between 18 triggers and 9 experiences. For example, if they felt that letters triggered colour, they would

draw a line between "letters" under triggers and "colour" under experiences (see appendix C for a full list of triggers and experiences). Participants were also given space to indicate any other synaesthetic associations they may experience outwith the common inducers listed on the questionnaire. Eleven patients initially self-reported some form of grapheme-colour synaesthesia (i.e., they linked the trigger "letter" or "number" with the experience "colour"). One subject was subsequently removed because further questioning revealed she had misunderstood the purpose of the questionnaire and in fact had no colours to report. This left 10 participants for further testing with a consistency test.

8.2.1.5 Consistency testing

As noted previously in Chapter 1, verification of synaesthesia relies on the behavioural gold standard test for synaesthesia, which assesses the consistency of the synaesthetic report over time (e.g., Simner et al., 2006b; Eagleman et al., 2007). For example, in the variant of interest here - grapheme-colour synaesthesia - the consistency test would first elicit the synaesthete's colours for each letter or digit, and then repeat this test a second time, to compare how consistently the subject reports their colour associations. Each of the 10 participants who indicated they experience grapheme-colour synaesthesia was therefore recontacted with the aim of assessing whether the synaesthesia they initially reported could be considered genuine, according to its consistency.

I assessed consistency in two ways: using either a short term or long term consistency measure. The short-term consistency measure was the single session test described in detail in Chapter 6, and this relied on the online assessment at www.synesthete.org, known as the Synesthete Battery (Eagleman et al., 2007). This presents each grapheme three times in a random order and requires subjects to select their synaesthetic colour from an on-screen colour palette. The test then assesses how consistently colours are selected for each grapheme (see Chapters and 6 for further details of this methodology).

The longer term retest was based on methods by Simner et al. (2006b) and was carried out as follows. The self-declared grapheme-colour synaesthetes were given a list of 26 letters and 10 digits in a blocked design and asked to report verbally or in writing their synaesthetic colour for each grapheme (where possible, the grapheme-colour associations for each participant were recorded at the time he/she completed the initial screening, at the point I collected the questionnaire from them in the clinic). Once a significant period of time had elapsed since completing the initial screening questionnaire, typically a minimum of 3 months (M = 6.6 months, SD = 3.8 months), the participant was recontacted without warning by telephone or email and asked to report again their colour associations for each grapheme. If the occasion presented itself to retest a participant on a subsequent appointment at the clinic (e.g., a six month follow up appointment), this opportunity was taken.

In summary, some patients were given the short term retest (Eagleman et al., 2007) and some the long term retest Simner et al. (2006b). The choice of retest method from patient to patient was determined by a variety of factors. Some participants did not have access to the internet or have a computer on which to carry out an online test, thus precluding the short term test. An additional consideration was the physical and cognitive condition of the participant. Multiple sclerosis is a heterogeneous condition that affects individuals in a wide range of different ways (see Chapter 3). Some subjects experienced either physical or cognitive impairment to the extent that online testing was not possible to conduct. Again, as a consequence, the long term test was administered and again therefore, both retest methods were required for this population. The results from Chapter 6 indicate that short term and long term testing produce comparable outcomes, and can therefore be considered equivalent in the identification of grapheme-colour synaesthesia.

8.2.2 Results

8.2.2.1 Synaesthesia prevalence

In this section, I calculate the prevalence of synaesthesia in MS patients to compare with the rates expected in the general population. Specifically, I calculate two measures of prevalence. First I count synaesthetes as all those who self-declared the condition. Then I count all those who were ultimately verified as genuine in a consistency test. I report both these measures because they tend to differ significantly. For example, it has previously been shown (Simner et al., 2006b) that for every six synaesthetes that self-report having synaesthesia, only one is consistent enough to be considered a genuine synaesthete. These two measures can therefore provide different baseline comparisons and we shall see below that the usual types of consistency tests may not be equally applied to healthy versus clinical populations (see general discussion in Chapter 10). These prevalence rates were then compared with the baseline prevalence rates found in the general population, screened via the online test reported in Chapter 6.

8.2.2.2 Self-reported prevalence

In the prevalence study carried out using a sample of the general population and reported in Chapter 6, 4.9% of participants self-declared experiencing grapheme-colour synaesthesia. In this study, 3.7% of MS patients self-declared experiencing grapheme-colour synaesthesia. This difference is not significant ($\chi^2 = 1.768$; $df = 1$; $p = 0.18$). Of the 11[1] MS patients (out of 300) who self-declared grapheme-colour synaesthesia, 9 were female, and their mean age overall was 47.5 years old (range 27-77, SD 14.4). Six subjects reported experiencing colour with letters only, three subjects reported experiencing coloured numbers only and two subjects reported experiencing both coloured letters and numbers. Details of all self-reported grapheme-colour synaesthetes are shown in table 8.1 below.

[1]Here, I include the 11 participants initially self-declaring grapheme-colour synaesthesia, rather than the 10 participants ultimately considered for consistency testing. This follows the methodology employed by the baseline study of Simner et al. (2006b)

	Grapheme variant				
Sex	**letters**	**both**	**numbers**	**Test interval**	**Retest method**
F	X				
F		16/36		11 months	Long-term
F	X				
F	17/26			4.25 months	Long-term
F	X				
F		1.09			Short-term online
F	X				
F			10/11	4.5 months	Long-term
F			0.51		Short-term online
F			X		
M	X				

Table 8.1: **Details of MS patients self-reporting with grapheme-colour synaesthesia. This table shows the variant of grapheme-colour synaesthesia experienced by the subject and the consistency score obtained in the test. The consistency scores are either the Eagleman consistency score (single number format, where a score of < 1 = synaesthesia) or the number of correct graphemes obtained when retested (out of 26 if letters only, 10 if numbers only and 36 if the subject reported experiencing both). The test method is indicated, and in cases where a test-retest method was used, the interval between testing is given. An 'X' denotes a participant who was approached for retest but did not complete a test**

8.2.2.3 Verified prevalence

As noted above, 10 of the 11 subjects who self-declared experiencing grapheme-colour synaesthesia were considered for retesting to objectively assess the consistency of their synaesthetic experience. Five participants successfully completed a test of consistency. Two completed the online test at www.synesthete.org (Eagleman et al., 2007) and three were tested using the test-retest methodology (Simner et al., 2006b).

Of the remaining five patients, one was unavailable to be contacted for retest, despite repeated efforts to make contact. The other four participants were contacted for retest but were unable to provide any colour associations at all at the time of their retest. Their reasons were highly similar in that all reported not being able to recall their colours anymore at the time of the retest. Colours were described as either too

vague, too changeable or entirely forgotten. Of the five subjects who sucessfully did a retest, two met the required consistency criteria to be considered genuine synaesthetes, thus giving a verified prevalence of grapheme-colour synaesthesia in multiple sclerosis patients of 0.67%. This prevalence is not significantly different from the baseline prevalence of 1.2% reported in Chapter 6 (Fisher's exact test, $p = 0.57$).

8.2.2.4 Bayesian analysis

To further investigate whether our result provided evidence in support of the null hypothesis (that having multiple sclerosis does not influence the likelihood of developing synaesthesia), I calculated a Bayes factor. By comparing the likelihood of two models (in this case, the null and alternative hypotheses) as a ratio, Bayes factors allow us to evaluate to what extent the data supports the hypothesis under investigation (Rouder et al., 2009). Following Jeffreys (1961), a Bayes factor of less than 0.33 provides strong support for the null hypothesis, a Bayes factor of greater than 3 provides support for the alternative hypothesis and values in between indicate the data are insensitive and no firm conclusions should be drawn. I calculated a Bayes factor of 0.019, indicating that having multiple sclerosis does not significantly increase the likelihood of having grapheme-colour synaesthesia.

Modelling our experimental data allows us to gain further insight into the influence of multiple sclerosis on synaesthesia prevalence. Constructing a beta-binomial model of our acquired data, we can show that there is an 30% chance that the MS prevalence of synaesthesia is higher than the baseline prevalence. If I calculate a 95% confidence interval of the difference in prevalence, this shows that any difference in prevalence between MS patients and healthy controls is likely to fall in the range -1.15% to 1.22%. To illustrate this with a theoretical example, if we were certain our baseline prevalence of 1.2% was correct, we would be 95% sure that the true prevalence of grapheme-colour synaesthesia in MS patients would be in the range 0.05% [1.2%-1.15%] to 2.42% [1.2%+1.22%].

8.3 Conclusions

This study investigated the prevalence of synaesthesia in a population of multiple sclerosis patients. Limiting the subtypes of synaesthesia to the single subtype of grapheme-colour synaesthesia, a prevalence of 0.7% was discovered. Contrary to my initial hypothesis, this prevalence is not significantly higher than the rates of synaesthesia found in a non-clinical sample of the general population, reported in the baseline prevalence study conducted in Chapter 6. In addition to the possibility that there is indeed no increased prevalence of synaesthesia in MS patients, there are several issues which may have affected the result of this investigation. These are discussed in greater detail below.

Throughout the synaesthesia literature, genuine synaesthetes are identified based on the consistency of their associations (see Simner, 2012 for a review). Because this method of identifying synaesthetes is so central to the literature, it was the logical approach to follow in this study. However, the outcome of the current experiment has highlighted one key problem with testing MS patients, namely that the cognitive impairments that are symptomatic of this condition make it highly probable that this patient group are unlikely to be as consistent as healthy members of the general population, even if they are genuine synaesthetes. Normally when conducting experiments, the experimenter tries to control all variables apart from the independent variable. Put another way, the researcher aims to compare like with like, as much as possible. In this study, I am comparing two samples that differ significantly in their clinical profiles and as a consequence, are likely to differ in their ability to complete tests of consistency, as we have now learned. For a variety of reasons, fewer than half of the participants who initially reported experiencing grapheme-colour synaesthesia completed any form of retest. Four of those failures resulted from memory problems, synaesthetic associations fading with age or changing with mood; problems that are likely to be due in part to having multiple sclerosis. To summarise, the problem is that the majority of studies aiming to identify synasthetes use consistency of association between inducer and

concurrent to do so. Clinical populations - such as those with MS in the current study - often have significant cognitive impairments that make them likely to be less consistent,whether they have synaesthesia or not. I will return to this issue in the general discussion (see Chapter 10).

This discussion above raises a second issue: should this potential difference in performance across populations have been identified in advance, and effectively precluded our particular choice of methodology? It is certainly true that the cognitive limitations of MS patients are well documented in the literature. Cognitive impairment is common in MS, experienced by approximately 50% of patients, in both early and late stages of the disease (Benedict et al., 2006; Rao et al., 1991). Particularly affected are processing speed, visual learning and memory, with upwards of 50% of patients experiencing deficits in these specific domains (Chiaravalloti and DeLuca, 2008). It is clear therefore, that MS patients experience significant cognitive impairment relative to the general population. However, there are two arguments for nonetheless planning a consistency test in a study aiming to verify MS synaesthetes. The first is that the burden of cognitive impairment experienced by MS patients can vary significantly. MS is a highly heterogeneous condition and while some patients do experience more cognitive problems, others experience more physical impairments, leaving them theoretically able to perform a consistency test like any other potential synaesthete (so long as their interface is adaptable, as it was here). In addition, MS has a distinct clinical profile, in that sufferers can experience clinical episodes that greatly impact many aspects of their functioning during the episode, but then they recover and their impairments dissipate. Again therefore, a consistency test might well have been suitable if our participants were able to complete it while in a recovery period (or if I had been able to select patients that had physical rather than cognitive deficits only). Nonetheless, the rarity of MS meant that I was ultimately forced to test all MS patients that became available, and this therefore included a number that were in later chronic stages, and or cognitively impaired. The later stages of MS result in a phase in which

the relapse-remit profile of the illness earlier on is replaced by a more chronic stage, in which the condition of the patient deteriorates without returning fully to their previous cognitive and/or physical capacity (Herndon, 2003). This fact will have made a great difference in our participants capacity to complete the type of consistency test described above. For all these reasons therefore, MS will have contributed to a significantly reduced capacity for consistency of response, and this is perhaps attested to by the fact that four out of 10 participants had cognitive difficulties that prevented them from completing my retest for consistency.

A second consideration is that, at the time of testing, the prevailing literature suggested that synaesthetic associations may be more similar to percepts rather than memories. If so, it would be reasonable to assume that cognitive losses (e.g., in memory) might nonetheless allow for MS patients to perform a consistency test unimpeded. For example, Kim and Blake (2013) describe a range of perceptual qualities to synaesthetic colours in their comprehensive review, and Simner and Logie (2008) presented early evidence to suggest that synaesthetic sensations may be exempt from some of the usual limitations of memory. However, it is unlikely that synaesthesia occurs in the absence of input from memory and indeed, synaesthetes have been shown to demonstrate improved memory in certain domains (Rothen et al., 2012). The responses of our impaired population seem to add weight to the suggestion that memory is an important element in the generation of synaesthetic associations. Memory loss happens gradually to some extent in the population in general, but MS patients are particularly vulnerable to such memory impairments (Chiaravalloti and DeLuca, 2008). Because of these factors, direct comparisons between MS patients and the general population on measures of consistency are difficult to make if these involve memory recall - a synaesthete with MS is perhaps simply likely to be less consistent than an unaffected synaesthete.

Aside from MS, the samples tested from the general population (i.e., in Chapter 6) and in the prevalence study conducted by Simner et al. (2006b) differed from those in

this study in other ways too, and this might also have resulted in a lower prevalence of synaesthesia in our sample of MS patients. The mean age of participants in Chapter 6 was 28.6 years, in Simner et al. (2006b) all the participants in one key study were undergraduate students, so the mean age would almost certainly be in the low twenties (age data are not reported). In contrast, the mean age in my current study was 41.1 years (of all participants) and 47.5 years (of self-declared synaesthetes). There is evidence that the consistency of synaesthetic associations decreases with age. For example, Meier et al. (2014) found age related changes in synaesthesia which suggests consistency of colour associations might decrease with age. This age difference across studies may also be a contributing factor to the lack of difference found between healthy and MS samples. It is possible the prevalence of synaesthesia in a young (i.e. matched to the baseline) sample of MS patients may be higher than in the older sample tested here.

What possible solutions could be employed by researchers designing studies seeking to compare synaesthesia prevalence in clinical and non-clinical populations? There are several potential approaches that may be utilised for future studies. One option might be to increase the accepted threshold for consistency when considering the clinical group being tested. In other words, use a less stringent level of consistency when assessing synaesthesia in the clinical sample. In Chapter 6, I replicated the most widely used online test for synaesthesia, produced by Eagleman et al. (2007). This test produces a quantitative score of consistency, in which a participant scoring < 1 is considered to be a synaesthete. If there was a reliable way of establishing the burden of cognitive impairment (e.g., by conducting a standard test of cognitive function) experienced by the participant at the time of their testing, the threshold at which they would be considered consistent enough to be a synaesthete could be adjusted. Unfortunately, there are several problems with this solution. Firstly, it requires the subject to complete an additional test (i.e., tests of cognitive function), which increases the physical and cognitive burden on the participant during testing. Secondly, there is no guarantee

that a given reduction in cognitive capacity would translate exactly to a parallel loss in consistency. For example, if it could be established that a potential synaesthete was suffering a 15% impairment in cognitive function, there is no understanding if whether this deficit would map to a corresponding 15% reduction in consistency. An additional approach could be the recruitment of a test population that would be expected to have an increased prevalence of synaesthesia relative to the general population. Given that MS and synaesthesia are both hereditary to some degree, measuring the prevalence of synaesthesia in first degree relatives of MS patients could be an alternative approach to investigating this association, which would allow the recruitment of a larger sample of participants. If the prevalence of synaesthesia was higher in this group, this could provide evidence of a link between synaesthesia and MS.

In this chapter, I conducted an investigation into the prevalence of grapheme-colour synaesthesia in a sample of patients with MS. The results of this investigation do not provide evidence of any association between MS and synaesthesia. The prevalence of grapheme-colour synaesthesia in this sample of MS patients was not significantly different from the prevalence found in the general population, as reported in Chapter 6 and elsewhere (Simner et al., 2006b). Cognitive and physical impairments experienced by the patients limited their ability to complete the tests required to objectively verify the genuineness of their synaesthesia, making direct comparisons between these groups difficult. I discuss the implications of this result in the context of this thesis as a whole in Chapter 10. In the following chapter, I turn to other possible comorbidities with synaesthesia, and present an empirical and theoretical link to one particular condition: anxiety disorder.

Chapter 9

Synaesthesia and other comorbidities: A health screening approach

9.1 Introduction

In Chapter 2, I provided an overview of the current research into synaesthesia and comorbidity. As this literature review shows, research into synaesthesia and potential associations with clinical conditions is in its infancy. The majority of studies in the literature report single case studies, with only a small number of investigations providing group level comparisons. In this chapter, I report empirical work in which a large sample of the general population completed an online test for synaesthesia (as first reported in Chapter 6) and also completed a health questionnaire. This generated a cohort of randomly sampled synaesthetes, each giving a full description of the state of their health. Within this sample, I examined four conditions in particular (anxiety disorder, asthma, hay fever and irritable bowel syndrome) to evaluate whether their prevalence was higher than would be expected in the general population see below for why these conditions were tested in particular). My results show that the prevalence of anxiety disorder is significantly higher in these randomly sampled synaesthetes in comparison to the general population, and that being diagnosed with anxiety disorder can actually influence the nature of the synaesthetic experience. Specifically, it

decreases the luminance of the synaesthetic colours perceived by the synaesthete. Sections of this chapter have been published in the form of a journal article, as Kay et al. (2014) (see Appendix H).

As noted above, the key aim of this study is to record data on the prevalence of a range of clinical conditions and ascertain whether any of them are more prevalent in grapheme-colour synaesthetes. Previous work has shown an association between synaesthesia and several conditions (see Chapter 2) and most relevant for the current chapter is that these include a link between positive schizotypy (personality features conferring increased risk of developing schizophrenia, as per Meehl, 1990) and synaesthesia (Banissy et al., 2012a). Because experiencing anxiety is strongly linked to the expression of positive schizotypy (Debbané et al., 2009; Lewandowski et al., 2006), the first hypothesis under investigation in the current chapter is that the prevalence of anxiety disorder would be expected to be higher in synaesthetes.

In addition, my current study sought to further investigate possible links between synaesthesia and immunity. In Chapter 5, I put forward the immune hypothesis of synaesthesia. This hypothesis proposes that the altered cortical connectivity thought to be a causal factor in the origins of synaesthesia arises as a result of genes that are known to have immune system functionality in the adult brain but act upon the mechanisms of cortical connectivity during development. As a consequence, clinical conditions known to be linked to the immune system were of particular interest in this present study. For this reason, a limited subsection of conditions listed on a health questionnaire completed by our testing population in the current chapter were selected *a priori* for further analysis. These conditions were: anxiety disorder, hayfever, asthma and irritable bowel syndrome (IBS), the latter in particular because of a previously reported finding in a group of IBS patients, by Carruthers et al., 2012. These immune linked conditions - along with anxiety disorder - were compared between the synaesthete and non-synaesthetes, identified by my wide-scale screening for synaesthesia.

The third aim of my current study was to explore whether any of the conditions found to be more prevalent in synaesthetes affected the synaesthetic experience in any way. To do this, I compared the characteristics of the colours chosen by synaesthetes with versus without the condition under investigation (e.g., I compared synaesthetes with and without anxiety disorder to see whether their synaesthetic colours were affected by this diagnosis). There are several reasons to think there might be such a difference. Although there is virtually no experimental evidence showing that health conditions can affect the colours experienced by synaesthetes, there are sporadic anecdotal reports in the literature pointing this way. For example, Day et al. (2013) describes in detail how his synaesthesia temporarily faded after he experienced post-traumatic stress disorder, and Cytowic (1995) discusses a case study in which a synaesthete with epilepsy reported that taking her anti-epilepsy medication made her synaesthesia less vivid. Finally, synaesthetes self-reporting for study in our lab have stated that their colours paled or disappeared during treatment for cancer (Julia Simner, personal communication) and other conditions such as MS (as reported in Chapter 8). For this reason, it is possible that clinical conditions might affect the nature of synaesthetic colours, and we explore this in the current chapter.

Finally, there are *a priori* reasons to think that anxiety disorder, in particular, may affect synaesthetic colours in systematic ways. First, emotion is known to be a synaesthetic inducer in its own right (Ward, 2004) suggesting that it can play a role in mediating synaesthetic experiences. Second, Dael et al. (2013) have hypothesised that negative mood states may diminish the intensity of synaesthetic experiences, even if those experiences are not triggered by mood *per se*. In other words, it is possible that synaesthetes with anxiety disorder may have notable changes to their synaesthetic experience, even if that experience is triggered by non-mood related inducers (e.g., graphemes). Furthermore, it is possible that changes in mood and emotion can influence colours indirectly because they have an impact on cognitive processes such as attention (Compton, 2003; Wadlinger and Isaacowitz, 2006). Attention has been shown

to play a significant role in the modulation of the synaesthetic experience (Rich and Mattingley, 2013), which may lead to mood affecting synaesthesia indirectly. There is also evidence suggesting clinical conditions may influence the perception of colour more directly. For example, Bubl et al. (2010) compared retinal activity in patients with depression, and found that patients had reduced contrast perception, relative to healthy control subjects. Taken together, this evidence suggests that the presence of a clinical condition may exert some influence on how a synaesthete perceives their synaesthetic colours.

9.2 Experiment 5

In this study, a large sample of randomly recruited participants completed a health questionnaire and an online test, screening for grapheme-colour synaesthesia. From these data, the health profile of the whole sample was obtained, and the randomly sampled grapheme-colour synaesthetes within this sample were identified. Thus, the prevalence of certain selected clinical conditions between synaesthetes and non-synaesthetes could be compared.

9.2.1 Methods

9.2.1.1 Participants

Participants took part in both the study described in Chapter 6 and the current study as part of the same testing session, described previously (Chapter 6). As such, the same 2847 participants took part in this study (1317 male, 1530 female; mean age 28.6, range 16-90, SD 14.3), and the recruitment method for this study is exactly the same as described in Chapter 6.

9.2.1.2 Materials/Procedure

As described in Chapter 6, randomly recruited participants were sent to an online testing site where they completed two tasks in sequence. The second of these tasks was

a screening for grapheme-colour synaesthesia, and this was exactly as described in Chapter 6. The first of these tasks, newly introduced here, was a health questionnaire. This was a one-sheet online form which began "Have you ever been diagnosed with any of the following conditions?" Subjects were asked to tick a box for any of the 24 conditions that applied to them (see figure 9.1 for these conditions). This yes/no measure allowed point estimates of prevalence to be collected for each condition for all participants, before they were required to give any information regarding whether they experienced synaesthesia or not. The conditions were selected on the basis of being prevalent in the general population and were laid out randomly in the questionnaire. The four target conditions were anxiety disorder, asthma, hay fever and IBS. These conditions were selected on the basis of being either previously linked to synaesthesia in the literature (which was the case for anxiety disorder Banissy et al., 2012a and IBS Carruthers et al., 2012) or by being prototypical immune conditions whose prevalence is sufficiently high in the general population to obtain high numbers in a sample of the size obtained in this study.

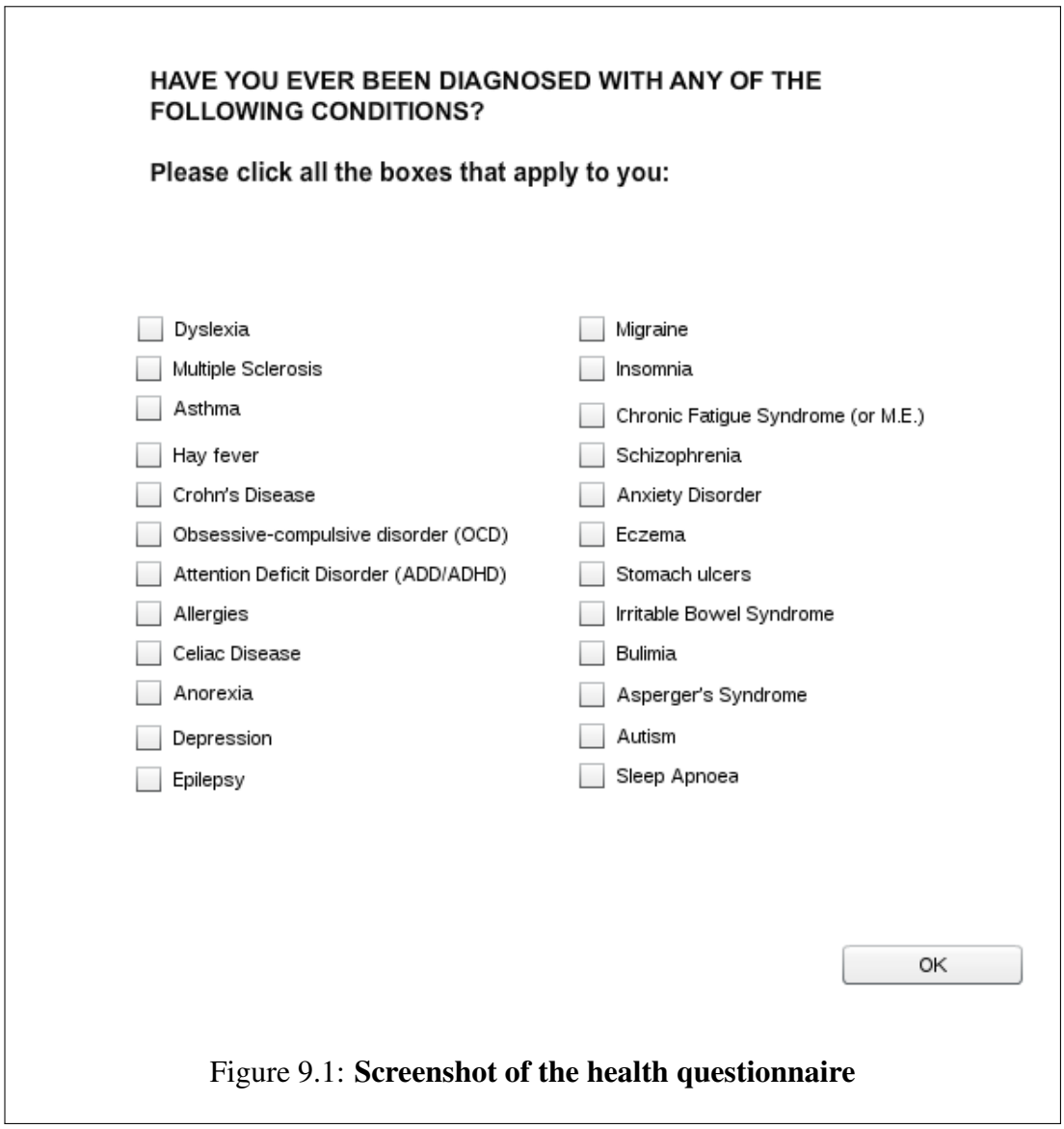


Figure 9.1: **Screenshot of the health questionnaire**

9.2.2 Results

In this results section, I first briefly review the findings of the grapheme-colour screening test (i.e., how many participants were diagnosed with synaesthesia) and then I relate this finding to the health questionnaire. In particular, I report the prevalence of each of our target conditions (asthma, hay fever, anxiety disorder and IBS) within the population of people diagnosed with synaesthesia, compared to the remaining non-synaesthetes. Note that although there were 24 different conditions in the questionnaire, I report only the prevalence of four conditions, whose choice was scientifically motived in advance (see materials/procedure). In order to correct for multiple com-

parisons, I applied a Bonferroni correction, thus giving a 2-tail alpha level of 0.0125 (0.05/4).

As reported in Chapter 6, from the sample of 2847 participants, 34 subjects achieved a score of < 1 on the consistency test, which is the criterion used by Eagleman et al. (2007) to identify genuine synaesthesia. This places the prevalence of genuine grapheme-colour synaesthesia at 1.2%. I now consider each of our target health conditions in turn, since the first aim of this study was to ascertain whether the prevalence of any of the clinical conditions listed in the health questionnaire was higher in synaesthetes.

9.2.2.1 Synaesthesia and anxiety disorder

Of the 34 genuine synaesthetes identified by the screening test for grapheme-colour synaesthesia, 6 reported being diagnosed with anxiety disorder, giving a prevalence of anxiety disorder in synaesthetes of 17.6%. From the subjects who were classed as *non*-synaesthetes, 139 people reported a diagnosis of anxiety disorder, giving a prevalence of 4.9%, in line with previous estimates of between 4.3% and 5.9% found in the general population (Tyrer and Baldwin, 2006). Finding prevalence estimates in our non-synaesthete sample that are equivalent to accepted measures in the general population indicates that the self-reporting methodology used is this study is valid.

The prevalence of anxiety disorder was significantly higher in synaesthetes when compared to non-synaesthetes, using a two-tailed difference between independent proportions test, (p = 0.0028, z = 2.993). This significance value survives the smaller alpha specified by the Bonferroni correction. No significant difference in prevalence was found in any of the other conditions of interest; hayfever (p = 0.067, z = 1.83), asthma (p = 0.847, z = -0.193), and IBS (Fisher's exact test, 2-tailed p = 1.0). The prevalence of each condition across samples are shown in Figure 9.2, below.

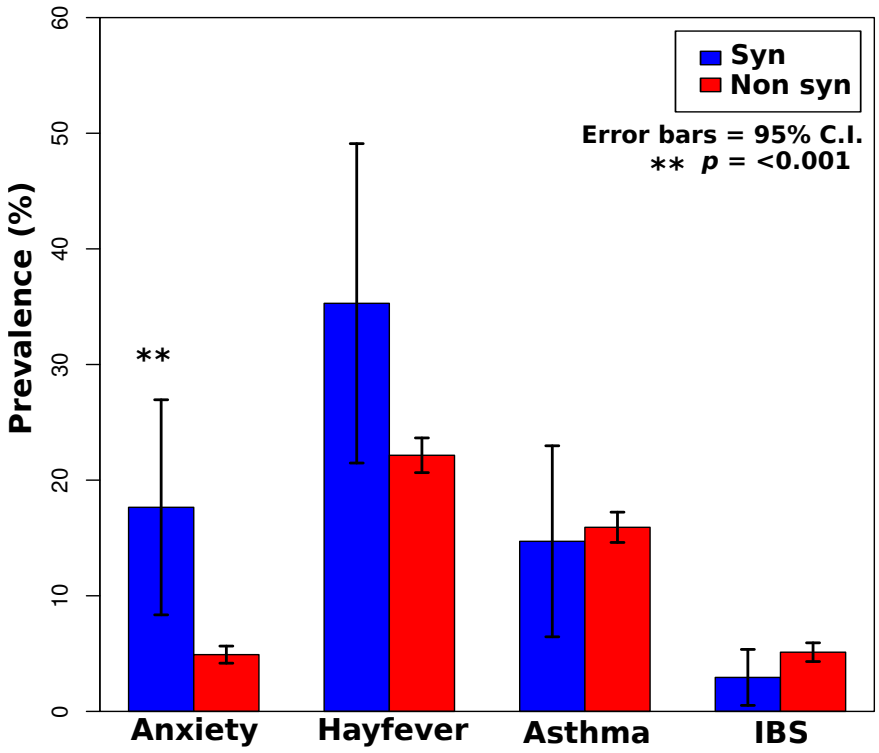


Figure 9.2: **Prevalence of selected clinical conditions in randomly sampled synaesthetes. Error bars are 95% confidence intervals**

9.2.2.2 Does anxiety disorder affect synaesthetic colours?

Above, I show that there are different prevalence rates of anxiety disorder in synaesthetes versus non-synaesthetes and I further explore this finding in the current section. In this analysis, I compared the grapheme colours of synaesthetes with and without anxiety disorder. As discussed in more depth in Chapter 6, the output of the online screening test for synaesthesia include not only an indication of consistency (i.e., how consistently colours are reported for each grapheme, which is the yard-stick for measuring genuine synaesthesia) but it also includes quantified information about the colours of graphemes encoded as RGB (red, green, blue) vector values. For this analysis, I converted these values to their corresponding hue, saturation and luminance values[1] . The average luminance of all graphemes for each subject across all their responses was calculated (maximally, this was a mean across all three presentations of

[1]This was done using an online conversion tool, found at http://www.rapidtables.com

each letter a-z and each digit 0-9, although participants were free to omit graphemes that had no synaesthetic colour. In other words, firstly a mean value was calculated per grapheme for each subject, then from those values, a single mean luminance value for that subject was calculated). This process was repeated with saturation values to also obtain mean saturation values for each participant. Note that I did not compare hue values since this is a circular dimension and cannot be compared in the same way (see Ward et al., 2006). Finally, I compared the mean luminance values for synaesthete participants with and without anxiety disorder and then compared their mean saturation values.

The saturation of synaesthetic colours was not significantly different for synaesthetes with anxiety disorder (mean = 209.4, SD = 12.6) compared to those without (mean = 191.0, SD = 35.9; t=1.2, df= 32, p=.2). However, synaesthetes with anxiety disorder had significantly darker (i.e, less luminant) colours (mean = 102.3, SD = 21.5) than those without anxiety disorder (mean = 121.9, SD = 20.4; t=-2.1, df= 32, p=.04, d=.89)[2] . This difference is shown in Figure 9.3 below.

[2]In order to further investigate the interaction between the presence of anxiety and the luminance and saturation of graphemes, a MANOVA was carried out. The result of this test was non-significant (F(2,31) =1.9, p=.17; Wilk's Λ = .89, partial η^2 =.11)

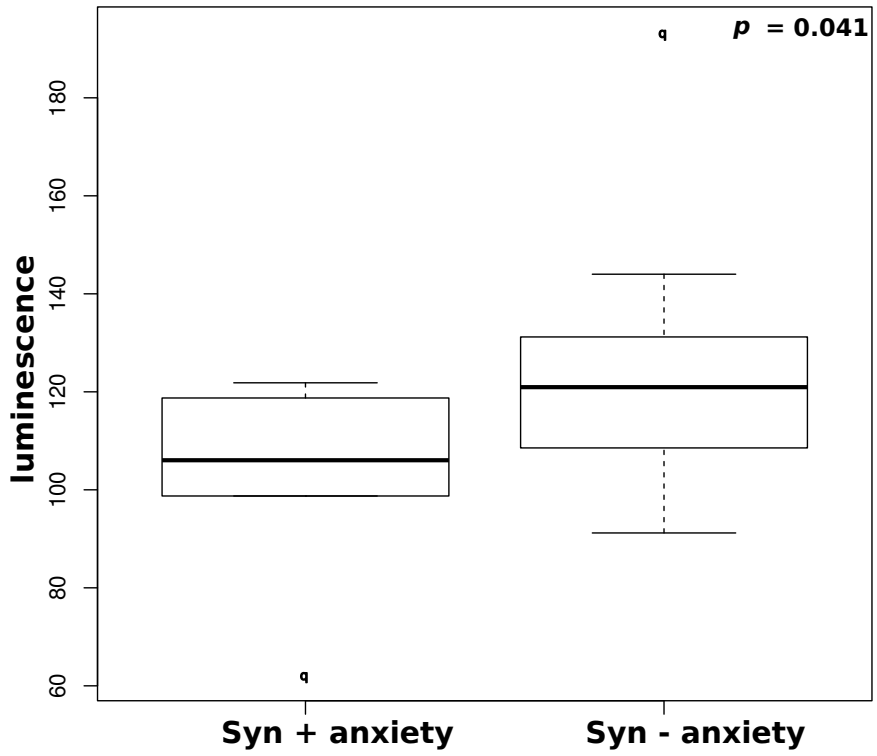


Figure 9.3: **Synaesthetes with anxiety disorder have coloured graphemes with lower luminance**

9.2.3 Discussion

In this study, I screened a large sample of the population for grapheme-colour synaesthesia in order to identify the small number of genuine synaesthetes within this group. As part of this online test, participants were also required to complete a health questionnaire in which subjects were asked to self-report having been diagnosed with a wide variety of clinical conditions. I found that the prevalence of anxiety disorder (but not asthma, hay fever or IBS) was significantly higher in synaesthetes in comparison to non-synaesthetes. Given the strong link between anxiety disorder and schizotypy (Debbané et al., 2009; Lewandowski et al., 2006), this finding supports previous work showing a link between positive schizotypy and synaesthesia (Banissy et al., 2012a).

Furthermore, the colours chosen by synaesthetes with anxiety disorder were significantly lower in luminance than those selected by synaesthetes without anxiety disorder. This finding supports existing evidence that mood and colour are related in

non-synaesthetes (Bubl et al., 2010). It might also be the case that mood can affect colour indirectly, by acting on cognitive processes such as attention (Compton, 2003; Wadlinger and Isaacowitz, 2006). It should be noted that although the prevalence of anxiety disorder in synaesthetes was high, the numbers of subjects with both synaesthesia and anxiety disorder are small, and as such, caution should be taken in the interpretation of these results.

I did not find significantly higher numbers of synaesthetes with any of the other autoimmune conditions selected for further analysis (asthma, hay fever or IBS). Previous studies in the literature have reported a higher prevalence of synaesthesia in IBS patients (Carruthers et al., 2012), but my current study did not detect an increased prevalence of IBS in synaesthetes. There are several reasons why this could be the case. Firstly, our number of genuine synaesthetes was small, and as such, this sample may not have been large enough to provide an accurate representation of the true prevalence of IBS in synaesthetes. The advantage of this study is that it avoided a self-referral bias and used only randomly sampled synaesthetes. Nonetheless, a consequent disadvantage was that the total number of synaesthetes was overall small. Given this, the number of synaesthetes with any given condition was also small, making it difficult to draw strong conclusions from null results. Nonetheless, I was able to show a significant effect in the case of anxiety disorder, and then show the consequences of having anxiety disorder on synaesthetic colours.

Another reason why I failed to replicate a link between synaesthesia and IBS (Carruthers et al., 2012) may relate to the age of the sample in the current study. The mean age of the sample participating in this study was 28.6 years old. Whereas the symptoms of IBS can occur at any age, patients usually present to a health care professional with IBS symptoms for the first time between the ages of 30-50 (Quigley et al., 2009). Indeed, the mean age of IBS patients in the study by Carruthers et al. (2012) was 43.1 years. Given the mean age of the sample in my study was under 30, a significant number who will ultimately be given a diagnosis of IBS at some stage in their life, will not

yet have received this. Therefore, the overall prevalence of IBS in this sample is likely to be lower than in the general population, which may have affected the number of IBS patients in the synaesthete group.

A further reason for failing to replicate the IBS link reported by Carruthers et al. (2012) may be the directionality of the association. Because Carruthers et al. (2012) found an increased prevalence of synaesthetes in IBS patients, the assumption that the association should hold in the opposing direction might not necessarily be true. This also mirrors other findings in this thesis. In Chapter 3, I report a higher than expected prevalence of MS-RIS in synaesthetes, but in Chapter 8, I did not find an increased prevalence of synaesthesia in MS patients (i.e., the same conditions but opposing direction of association). I discuss this further in the general discussion (see Chapter 10).

Chapter 10

Conclusions and future directions

10.1 Conclusions

In Chapter 1, I introduced the topic of synaesthesia, and discussed the neurology, development and causes of synaesthesia. I also reviewed some of the research questions pertinent to synaesthesia that have relevance for this thesis. Chapter 2 reviewed and summarised - for the first time - all the existing literature on conditions that may (or may not) co-occur with synaesthesia. In Chapter 3, I focussed on one particular condition, *multiple sclerosis (MS)* and presented data demonstrating that MS and its clinical precursor, *radiologically isolated syndrome (RIS)* are statistically more prevalent in synaesthetes self-referring to participate in scientific studies than in the general population. In Chapter 4, I considered the same question from a genetics viewpoint. I first provided a general introduction to the field of genetics,then a detailed review of the synaesthesia genetics literature and the genetics of multiple sclerosis. Next I explored why the two conditions may share common genetic origins. Chapter 5 presented my immune hypothesis of synaesthesia, which explores a theoretical framework explaining how the origins of synaesthesia may lie with genes that play an important role in the development of the cortex in the first years of life, yet also have an immune function in the adult brain.

In Chapters 6 and 7 I presented my own investigation into the genetics of synaes-

thesia which had the single aim of better understanding comorbidity by examining the roots of synaesthesia, and several related threads. These involved first establishing (in Chapter 6), a valid way to phenotype synaesthesia by evaluating an existing online testing platform (The Synesthesia Battery, Eagleman et al., 2007). This validation involved using the testing platform to generate the most accurate and large scale assessment of the prevalence of synaesthesia to date, and then comparing this to a previous, widely accepted prevalence figure established from a well-validated study using a different method (Simner et al., 2006b). My approach also provided the most accurate estimate of any sex bias within synaesthesia, which itself was used in my discussions of synaesthesia's aetiology and comorbidity (i.e., is synaesthesia more common in women? Is a sex bias found in any other potential comorbidities). In Chapter 7, I used this method of testing for synaesthesia to phenotype participants in my own investigation into the genetics of synaesthesia.

In Chapter 8, I presented data exploring the prevalence of grapheme-colour synaesthesia in a sample of patients with MS and in Chapter 9, I addressed a different area of comorbidity by presenting empirical work showing that anxiety disorder (but not asthma, hayfever or IBS) is more prevalent in randomly sampled synaesthetes when compared to non-synaesthetes. Finally, I showed a consequence of having both synaesthesia and anxiety disorder in that such synaesthetes experience less luminant colours compared to their synaesthetic peers without anxiety disorder.

In this final chapter, I will now summarise and evaluate the findings of the research conducted for this thesis, and I will discuss the strengths and weaknesses of my approach. The overall aim of this thesis was to explore comorbidity in synaesthesia, so I draw conclusions by considering each comorbidity in turn. First, I discuss what this work tells us about synaesthesia and MS, and second, what I have learned about the association between synaesthesia and anxiety disorder. In addition, examining synaesthesia and comorbidity subsequently led to the investigation of several methodological questions relating to synaesthesia research. I therefore also discuss these methodolog-

ical issues and the resulting outcomes further below.

10.1.1 Synaesthesia and MS

Perhaps the key question raised by this thesis centres on the question of comorbidity between synaesthesia and MS. When considering the findings across both my MS/synaesthesia prevalence studies (Chapters 3 and 8), the question is raised of how to interpret their combined findings. In other words, how do we interpret the fact that an unusually high number of MS/RIS cases were found when looking within groups of synaesthetes, but that synaesthesia was not found in high numbers when looking at patients with MS, and furthermore, that my genetics study found no conclusive link between the two conditions? One simple answer is that given the small sample of synaesthetes scanned in MRI studies (to date, N=243), these high rates of MS I found within the synaesthete population could have been a statistically spurious finding, despite the conservative assumptions adopted during my study (e.g., the calculation of conservatively established MS/RIS prevalence baselines). If this were the case, my other two findings (in genetics and MS patients) would have been as expected.

Nonetheless, there is a second possible answer to this question, and this relates to a methodological issue. In my conclusions to Chapter 8, I briefly discussed the difficulties in using the same synaesthesia diagnostic test (a test of consistency) for both the general population and for a clinical population of people with MS. I will elaborate on this issue towards the end of this chapter where I further consider the methodological challenges of this thesis. Until then, I remind the reader that the problem in particular is that clinical populations may be *a priori* unable to perform well in a consistency test - independent of whether they have synaesthesia or not. In other words, it is possible that our test for synaesthesia in Chapter 8 within MS patients was simply unable to produce robust findings, even if these patients had had synaesthesia in high numbers. This methodological consideration is all the more important when I consider the limitations of current knowledge in genetics studies too. In Chapter 5, I discussed a plausible the-

ory for why synaesthesia and MS may be related, based on the dual purpose of certain genes, which not only play an important role in synaptic pruning and thus the formation of cortical connections in early development (important for synaesthesia) but also have later life autoimmune implications (important for MS). Nonetheless, when I presented a genetic association study in Chapter 7, investigating whether genetic links could be found between synaesthesia, MS and immunity, there were no significant results. However, I concluded that chapter by discussing the limitations of the particular genetics approach I had used, specifically, the inability to control for population stratification effects (see Chapter 7). Because these limitations are overcome by carrying out a *genome wide* association study (GWAS), I conclude that genetic links between these two conditions can only be fully investigated upon completion of such a study. Although I and my collaborators are currently moving towards this goal (i.e., we are currently collecting far larger numbers of synaesthete DNA, as required by the GWAS approach), the GWAS study itself is outside the remit of this thesis. In summary, the conclusion to be drawn from my MS investigations in Chapters 3,6, 7 and 8 are that despite a plausible theoretical genetic link, and despite preliminary evidence that appears to show high rates of MS within synaesthetes, no other corroborating evidence has yet been found. My future GWAS genetics study might yet shed further light on this question.

10.1.2 Synaesthesia and anxiety disorder

In addition to investigating potential links between synaesthesia and MS, I also examined the prevalence of clinical conditions known to have either an association with immunity or which have been previously linked to synaesthesia in the literature; namely hayfever, asthma, IBS and anxiety disorder (see Chapter 9). In my study, I recruited randomly sampled synaesthetes in a large scale screening approach and then had all subjects complete a health questionnaire. Two findings emerged from my data. First, the prevalence of anxiety disorder (but not asthma, hayfever or IBS) was higher in synaesthetes than in non-synaesthetes. Second, the luminance of reported synaesthetic

colours was lower in synaesthetes with anxiety disorder when compared to synaesthetes without anxiety disorder. In other words, synaesthetes with anxiety disorder experience synaesthetic colours that are generally darker than synaesthetes without anxiety.

What conclusions can be drawn from these observations? In previous work linking synaesthesia and positive schizotypy, Banissy et al. (2012a) suggest that increased comorbidity with positive schizotypy points to the presence of synaesthesia as being indicative of broad differences in cognitive function and personality. Given the strong links between anxiety disorder and positive schizotypy (Lewandowski et al., 2006) and links between anxiety disorder and personality traits more generally (Bienvenu et al., 2004), my own findings lend support to that premise. With regards to comorbidity more generally, this brings a key, much broader, question into focus, namely the nature of the association between conditions. Is the link direct, or is the presence of synaesthesia and its comorbid condition both related to an additional, unidentified aspect of cognition, development or neurology. In Chapter 5, I suggest associations between synaesthesia and certain conditions such as MS may be related to genes responsible for both cognitive development and immune system function. Prevalence studies may not offer direct understanding into this aspect of comorbidity research but ultimately, the outcome of genetic studies into synaesthesia (see Chapter 7) may provide insight into the causal relationship between synaesthesia and its cormorbid conditions.

In Chapter 9, I showed that synaesthetes with anxiety disorder have less luminant (i.e., darker) synaesthetic colours. Previous evidence shows that mood can alter the perception of colour in non-synaesthetes. The mechanism by which this happens is still a question of debate. The presence of a clinical condition may exert an effect on the perception of colour directly, as has been shown in depression Bubl et al. (2010), or the effect may be modulated by an alternative cognitive process, such as attention (Compton, 2003; Wadlinger and Isaacowitz, 2006). The interesting point is that both non-synaesthetic and synaesthetic colour perception can potentially be influenced by

similar factors (i.e., the presence of anxiety disorder). This would therefore suggest that both synaesthetic and non-synaesthetic colours are influenced by the same perceptual mechanisms and may therefore originate via the same neurological pathways.

None of the other conditions I examined in the health questionnaire (IBS, hayfever or asthma) had an increased prevalence in the synaesthete sample. Several methodological differences that may have led to non-replication of the IBS finding are discussed in Chapter 9, but an additional point is worthy of further discussion here. Repeatedly, throughout this thesis, I refer to possible associations between synaesthesia and other conditions but without giving consideration to the question of whether or not that association would be bidirectional or not. For example, Carruthers et al. (2012) report a greater than expected prevalence of synaesthesia in IBS patients. One would assume that the association would hold in the opposing direction, namely that the prevalence of IBS should be higher in synaesthetes. My study in Chapter 9 did not find this outcome. Lack of replication was also found in my studies looking into synaesthesia and MS, but in the other direction (MS appears overrepresented in synaesthetes but not synaesthesia in MS patients).

10.2 Methodological advances and limitations

In Chapter 6, I screened a large sample of the general population for grapheme-colour synaesthesia. To do this, I replicated and evaluated the most commonly used online test for synaesthesia, the Synesthete Battery (Eagleman et al., 2007). This test is based on the behavioural gold standard diagnostic for synaesthesia: consistency (i.e., synaesthetes report their colours with greater consistency than non-synaesthetes). I showed that using an online test which screens for synaesthesia using a short-term consistency approach (i.e., in a single session) produces comparable results to studies (e.g., Simner et al., 2006b) that assess synaesthesia using long-term consistency (i.e., in two testing sessions with a significant time interval in between). My study found a grapheme-colour prevalence of 1.2% and no difference in the ratio of prevalence be-

tween the sexes. By replicating the grapheme-colour prevalence reported by Simner et al. (2006b), my result suggests that the single session methodology is a valid and reliable way of phenotyping genuine synaesthetes.

Previous studies ((Baron-Cohen et al., 1996; Rich et al., 2005) relied upon self-referral, and consequently found a large female skew of up to 6 female synaesthetes for every male synaesthete. When conducting my own study, care was taken to minimise self-referral bias. No-one could self-volunteer to take part in this investigation: all participants were individually approached to take part and it was only revealed that synaesthesia was the subject of the investigation once participants began the online test (see Chapter 6 for methods). In light of this improved methodological approach, the lack of difference in synaesthesia prevalence between the sexes indicates that previous differences reported in the literature (e.g., by Baron-Cohen et al., 1996) likely resulted from recruitment biases, rather than any other difference (such as sex-linked genetic reasons). My finding can therefore hopefully assist future research into the causes of synaesthesia by shifting focus from possible sex-based differences.

10.2.1 Screen or recruit? The referral bias dilemma

There is a methodological trade-off involved in the design of studies requiring the recruitment of participants with one or more rare conditions, and this concerns sample size versus self-referral bias. When dealing with (multiple) conditions that are rare in the general population, finding suitable participants is a challenge for researchers. Small samples can cause problems with statistical power, thus limiting the strength of conclusions that can be drawn from the presented evidence. On the other hand, because participants who go out of their way to volunteer to take part in research studies are *a priori* different from non-volunteers on numerous measures, such as level of motivation, level of education and openness to new experience (Rosenthal and Rosnow, 1975), we cannot be sure that a sample of participants who made the effort to self-volunteer to take part in a study are representative of the general population.

In Chapter 6 of this thesis, I took the approach of screening a large sample of the general population in order to identify the small number of genuine synaesthetes within that group, thus taking steps to minimise self-referral bias. Once this group of synaesthetes (n=34) was identified, I could then investigate questions such as the overall prevalence of grapheme-colour synaesthesia, its distribution between males and females and the prevalence of certain clinical conditions in synaesthetes. As noted above, the advantage of my study is that self-referral bias and (thus the confounding differences between volunteers and non-volunteers) is minimised. The disadvantage is that the number of synaesthetes is small. When considering issues such as the overall prevalence of grapheme-colour synaesthesia, or the bias in prevalence between the sexes, I consider my approach to be superior, in that it produced an accurate estimate of prevalence overall. Above therefore, I proposed that limiting recruitment bias will ultimately result in a more accurate prevalence estimate. An opposite viewpoint is also possible. For example, it would have been possible to advertise for grapheme-colour synaesthetes and recruit a larger sample than the one obtained here by screening the general population. As evidenced by different estimates of prevalence and sex bias in synaesthesia early in the literature (discussed in Chapter 1), the results would be significantly different using this approach.

10.2.2 Focus on grapheme-colour synaesthesia

In this thesis, I focussed on a specific synaesthesia subtype; grapheme-colour synaesthesia. There are several advantages and potential disadvantages to this approach that are worthy of discussion here. Grapheme-colour synaesthesia is one of the most common synaesthesia variants, with a prevalence of approximately 1.2% in the general population (Simner et al., 2006b). It is also one of the best understood variants, and is easy to test the consistency of grapheme-colour synaesthesia, relative to other subtypes (Simner, 2012). Grapheme-colour synaesthesia lends itself especially well to online testing that can be conducted remotely, which is a significant advantage when aiming to screen large samples of the general population.

The question remains however, can grapheme-colour synaesthesia be used as a proxy for all other forms of synaesthesia? If this approach is optimal, grapheme-colour synaesthesia should share the same neurological and genetic causes as other synaesthesia variants. Whether this is indeed the case remains an open question. In chapter 4, I refer to literature showing that certain subtypes co-occur in clusters (Novich et al., 2011), which lends support to the hypothesis that subtypes occur in clusters according to the inducer and/or concurrent in question. For example, synaesthesia subtypes with ordinal sequences (i.e., days and months) or graphemes as inducers and colour as an concurrent are statistically more likely to co-occur in the same person, whereas the likelihood of a grapheme-colour synaesthete experiencing lexical-gustatory synaesthesia is the same as that of a non-synaesthete (Novich et al., 2011).

It is possible that specific comorbidities may be more greatly associated with forms of synaesthesia other than grapheme-colour. For example, it is not unreasonable to propose that the prevalence of anxiety disorder may be higher in people who experience forms of synaesthesia linked to emotions, given the link between anxiety disorder and the regulation of emotion (Hofmann et al., 2012). It is possible therefore, that investigating anxiety disorder in only grapheme-colour synaesthetes may not provide a wholly representative picture of the prevalence of anxiety disorder in synaesthesia.

On balance however, I suggest the advantages gained from testing grapheme-colour synaesthetes are greater than the potential disadvantages. The issue of whether grapheme-synaesthesia is a suitable proxy for all synaesthesia subtypes only becomes relevant if indeed synaesthesia is an umbrella term for a collection of behaviourally similar conditions with different origins, and to date, this remains an open question. If this were indeed to be the case, it would limit conclusions from this thesis to be drawn with respect to grapheme-colour synaesthesia only, rather than allowing findings to be generalised to synaesthesia as a whole. The advantages however, are tangible. Grapheme-colour synaesthesia is well understood behaviourally, it is straightforward to test for, and lends itself well to online and remote testing. This enables large samples of the

size used in this thesis (in the thousands) to be screened for synaesthesia.

10.2.3 Comparing synaesthesia in healthy and clinical populations

As discussed above and in Chapter 1, this gold standard behavioural test for synaesthesia relies on consistency of response, either assessed across a significant long-term time interval or using short-term single session tests (e.g. Eagleman et al., 2007). Nonetheless, an important additional issue with studies comparing the prevalence of synaesthesia in clinical populations with the prevalence in groups of healthy individuals concerns how a fair comparison of consistency between groups might be established. In this thesis, I assessed the prevalence of synaesthesia in both healthy and clinical populations. This question is therefore especially pertinent, so I address it more fully below.

The fundamental question is this - do we expect the synaesthetes in either clinical or non-clinical groups to be equally consistent? Is this a fair comparison? Is it possible that synaesthetes with comorbidities may be *a priori* likely to be less consistent as a result of their additional condition (e.g., MS)? If so, how should researchers deal with this situation? The answer to this issue comes down to the question of why synaesthetes are consistent. Of primary concern is the possibility that only healthy synaesthetes may be fully consistent over time. Put differently, the test of consistency might be less appropriate in clinical populations, because their clinical condition may itself hinder consistency in reporting synaesthetic colours (or other sensations). This would of course depend on the type of comorbidity under consideration. One would imagine that when considering conditions without any significant impact on memory, cognitive performance or executive function (e.g., asthma), comparisons of consistency would be valid. It would however, be reasonable to assume that conditions with greater clinical burden (e.g., MS) could potentially reduce consistency. The problem then becomes a matter of interpretation. Does a low level of consistency mean the person should be considered less synaesthetic or that a reduction in general cognitive function

indirectly linked to synaesthesia is responsible? In other words, the presence of illness if a potential confound when assessing the consistency of synaesthesia. Furthermore, individual synaesthetes are likely to be impacted to varying degrees - for example, the consistency of every synaesthete with schizophrenia is not going to be affected to the same extent. Do we conclude that if the consistency of their synaesthetic responses is affected by the additional condition they suffer from, their synaesthesia is any less genuine? If it is the latter, the question of how this should be addressed is a significant one.

Several of the studies discussed in Chapter 2 highlight the methodological difficulties and potential discrepancies in results that may emerge when comparing clinical and non-clinical populations. For example, Baron-Cohen et al. (2013) report difficulties in getting autism patients to successfully complete tests of consistency, effectively preventing objective comparisons of synaesthetic consistency to be made between patients and controls. Carruthers et al. (2012) found IBS patients were less likely to meet increasing strict consistency thresholds relative to control subjects. When consistency was measured using thresholds of 50% accuracy and 75% accuracy, the number of IBS patient synaesthetes obtaining these levels of consistency dropped significantly, whereas the number of controls did not. This could have two possible causes; either IBS patients are somehow more likely to indicate they have synaesthesia when they do not, or the reduced consistency of their responses may be perhaps related to their illness. In my own work investigating the prevalence of synaesthesia in multiple sclerosis patients (Chapter 8), we saw that consistency of response in self-reported synaesthetes varied significantly, as did the burden of cognitive impairment experienced by some participants. I concluded that it was likely that cognitive problems contributed to a significantly reduced capacity for consistency of response.

I conclude this thesis by pointing to one final issue in the domain of consistency testing in synaesthesia research. While consistency has thus far been considered the defining benchmark of the synaesthetic experience (Baron-Cohen et al., 1993, 1996;

Mattingley et al., 2001; Rich et al., 2005; Simner et al., 2006b), recent evidence suggests that the link between synaesthesia and consistency may not be as rigid as expected. Simner (2012), made the point that establishing consistency as a defining criterion of synaesthesia leads to the identification of consistent synaesthetes only and the rejection of the rest. She points out however, that there may be a significant number of synaesthetes who genuinely experience automatic synaesthetic associations when presented with inducers, but simply happen to be non-consistent over time. In other words, Simner (2012) revises the possibility that consistency may be a false foundation on which to define synaesthesia: many synaesthetes are consistent but some may not be. One ideal step forward in the synaesthesia literature - and especially the literature that seeks to find comorbidities - might be to find new ways to identify synaesthetes, which may ultimately allow a fairer comparison across synaesthete and non-synaesthete populations.

Appendix A

Information sheet from MS prevalence study

Exploring the Connection between Synaesthesia and Multiple Sclerosis

PARTICIPANT INFORMATION SHEET

We would like to invite you to take part in a research study based at the University of Edinburgh and NHS Lothian. This study involves patients who have been diagnosed with Multiple Sclerosis (MS). Before you decide it is important for you to understand why this research is being done and what it will involve. Please ask us if there is anything that is not clear and take your time to decide whether or not you wish to take part.

1) What is the purpose of the study?

This project aims to investigate a potential link between synaesthesia and MS. Synaesthesia is a benign form of perception in which stimulation of one sense leads to automatic, involuntary experiences in a second sense. For example, perception of numbers and letters may be associated with the experience of colours or a musical note may be associated with a particular colour. The purpose of the study is to explore the connection between synaesthesia and MS, which may ultimately lead to a better understanding of the causes of both synaesthesia and MS.

2) Who is organising and funding the research?

The research is being organised by the University of Edinburgh. This project is being supervised by Dr Julia Simner and Professor Stephen Lawrie who work in academic research, in collaboration with the Department of Clinical Neurology, NHS Lothian.

WHY HAVE I BEEN ASKED TO TAKE PART?

This study requires the participation of MS patients and a group of healthy volunteers to act as control subjects. You have been approached either because you are an MS patient attending an MS clinic at the Western General Hospital or because you are accompanying a patient attending this clinic.

3) Do I have to take part?

No, your participation in this study is entirely voluntary. You are free to decline to enter, or to withdraw from the study at any time without having to give a reason. Participation in this study will not affect your ongoing care.

4) What will happen to me if I take part?

Participation in this study involves completing a questionnaire and taking part in an online behavioural test for synaesthesia. The questionnaire should take less than 20 minutes and the online test should take approximately 45 minutes. Both tests are designed to establish if you have any form of synaesthesia. Only participants who report experiencing synaesthesia will be asked to complete the online test, which can be quite time consuming and require concentration to complete. The online test requires access to a computer connected to the internet. This test maybe completed at home at a time convenient to you or alternatively, in the clinic, should you not have access to the internet at home. Completing the online test requires giving consent online and any data obtained will be stored on an external database any may be used in future by owners of the site. Should you be asked to complete the online test, you will be provided with an email address and login to allow this test to be completed anonymously.

5) Are there any risks or disadvantages if I take part?

There are no anticipated risks or disadvantages to taking part in this study.

6) Will taking part interfere with my treatment?

No, taking part will not affect your clinical care in any way.

7) What about my medication?

We would ask you to take your medication as normal. We are not testing a new treatment.

8) What are the potential benefits of taking part?

We do not anticipate any direct benefits to you. However, we hope to gain a greater understanding of whether synaesthesia and MS are connected. In doing so, it is possible that a better understanding of the causes of MS might be obtained.

9) What happens when the research study stops?

When the study finishes, we will analyse the data and determine the results. The results will then hopefully be published in scientific journals. If you would like to hear about the final results of the study, please let us know and we will arrange this with you. We will also retain your details about you as described below.

10) What information will be held about me?

Data from the questionnaire and online test will be collected, along with your name and date of birth. Questionnaires will be anonymised and coded with a unique identification number. This information will be treated as strictly confidential and will only be used for the purposes of scientific research. The results of these tests will be stored on password protected computers and your personal details will be stored securely. When the results of the study are published or presented to a third party, all data will be in coded form to preserve anonymity. Any information that could link the data back to you (such as your name and address) will be completely removed. Anonymised data from this study will be made available to the research team. It is possible that relevant data may be used by other researchers working within the University of Edinburgh for other ethically approved research projects but only after approval from the chief investigator. Any publications arising from this study will be fully anonymised and will be made available to you upon request. This study fully complies with the Data Protection Act 1998 and Trust data protection policies.

11) What if there is a problem?

If you have a concern about any aspect of this study, you should speak to the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this via the NHS complaints procedure. Details can be obtained from Patient Advice and Liaison Service at any of the hospital sites involved.

12) Does my GP need to be informed?

Because synaesthesia is not considered a clinical condition and this study does not interfere with your health care in any way, we will not inform your GP about your participation in this study.

13) What will happen if I do not want to carry on with the study?

You may withdraw from the study at any point.

14) Will I be followed up after the study?

Participation in this study would not affect your ordinary care and follow up.

16) Who has reviewed and approved the study?

An NHS research ethics committee has reviewed and approved this study. This is an independent group of people who ensure that the dignity, rights and safety of participants and researchers are protected. The study has also been given NHS R&D management approval.

17) If you would like further information from an independent advisor, please call or write using the contact details below.

Name: Dr Anna Williams

Job title: Research Fellow and Honorary Consultant

Work Address: The Queen's Medical Research Institute

47, Little France Crescent

Edinburgh

EH16 4TJ

Daytime telephone: 01896 826 624

Email: annacwilliams@yahoo.co.uk

18) If you would like more information or need to contact me after taking part, please call or write to me using the details below.

Name: Duncan Carmichael

Job title: PhD Researcher

Work address: room 3.34 Informatics Forum,

10 Crichton Street,

University of Edinburgh,

EH8 9AB

Daytime telephone: 0131 650 4415

Email: d.a.carmichael@sms.ed.ac.uk

Many thanks for reading this information pack. Our research depends entirely on the goodwill of potential participants such as you. We realise that it may not answer all your questions. Please think about participation carefully and feel free to ask us anything you wish.

Appendix B

Consent form from MS prevalence study

Exploring the Connection between Synaesthesia and Multiple Sclerosis

PARTICIPANT CONSENT FORM

Please confirm your personal details:

Name: _____

Date of birth: _____

Address: _____

Email: _____

Contact number: _____

Participant ID: _____

PLEASE INITIAL THE RELEVANT BOXES REGARDING PARTICIPATION IN THIS STUDY:

1. I confirm that I have read and understood the information sheet (version 1.2, 23/05/2012) for the above study and have had an opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason. Should I withdraw, my medical care or legal rights are not affected. ☐

3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by the study researchers and individuals from the Sponsor, regulatory authorities or from the NHS organisation, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4. I agree to storage of personal information in a secure, locked cabinet to which only the research members will have access to. ☐

5. I understand that anonymised data will be stored securely and processed in the research department. If appropriate, this data may be reviewed by future researchers after the study has ended. ☐

6. **I agree to take part in this study** ☐

Name of Participant: _____

Signature of Participant: _____

Date: _____

Name of Researcher: _____

Signature of Researcher: _____

Date: _____

Appendix C

Questionnaire from MS prevalence study

Prevalence Synaesthesia Questionnaire

What is synaesthesia?

- Synaesthesia is a benign form of perception in which there is a kind of 'cross-over' of the senses. So for example, for someone with synaesthesia, hearing music might give rise to the sensation of colour. There are many types of synaesthesia, and each one links some type of trigger (e.g., listening to music, reading numbers) with some type of crossed experience (e.g., a colour, a taste). Around 4% of people experience synaesthesia. People with synaesthesia very often do not realise that their experiences are different from other people – or they might view them as a simple eccentricity. For example, in one type of synaesthesia, people spontaneously associate letters of the alphabet with personality types or genders (e.g., A might be female, say, or untrustworthy). In another, they might feel that food tastes have different 'shapes' (e.g., chicken is pointed, banana is a small sphere). Other types of synaesthesia include letters triggering colour (r=dark red), words triggering food flavours (e.g., Philip = unripe oranges) or numbers, letters or time being 'seen' mapped out in space (e.g., months of the year in an oval shape). Of course, anybody could invent these types of association on the spot, but only people with synaesthesia will have had associations existing spontaneously, and before being asked in this questionnaire. These associations may date back since childhood or may have developed more recently.

What is NOT synaesthesia?

- Sensations triggering memories. E.g. a song reminding you of a person or place.
- Learned associations. E.g. the word *Christmas* being linked to the colour red or the sound of carols; the colour green being associated with envy.

The questionnaire below asks whether you any synaesthesia-like experiences. It also asks about other sensory experiences you might have. Please don't worry about first guessing whether or not you have synaesthesia – just give any information as accurately as you can.

SECTION 1: ABOUT YOURSELF

Date of birth: _____ **Sex:** Male ☐ Female ☐

Handedness: Right-handed ☐ Left-handed ☐ Ambidextrous ☐

Today's date: _____

SECTION 2: ABOUT YOUR EXPERIENCES

2A. **INSTRUCTIONS**: Please use the space below to tell us about your experiences. Please link the triggers on the left with your experiences on the right by drawing a line(s) on the questionnaire. For instance, if numbers give you the impression of colours, draw a line between *numbers* (which is the trigger, on the left) and *colours* (which is the experience, on the right). You may have no associations, or you may have one or more than one.

IMPORTANT: Please don't connect the same things (e.g. colours-colours) as this is assumed true of everyone. We also assume (without you having to indicate) that letters/words etc. are experienced as shapes on a written page, and musical instruments/voices/spoken words as noise.

TRIGGERS	**EXPERIENCE**
Letters of the alphabet	Colours
English words	Shapes/patterns
Foreign words	Tastes
People or People's names	Smells
Addresses/places	Pains/touches
Numbers	Noises
Days of the week	Flashes
Months of the year	Music/sound
Voices	Movement
Pains/touches	personality/gender
Movements/postures	
Music (instrumental)	
Noises	
Smells	
Tastes	
Colours	
Shapes/patterns	
Emotions	
Environmental sounds (e.g. a car engine, clapping hands)	

Other Associations? _____
_____
_____
_____

Some people experience sequences (e.g., numbers, days, months) in particular spatial patterns. For example, they might see months of the year in an oval shape, or a on a curved line. Does this apply to you?

- ○ No
- ○ Yes : Which sequences do you see? (please underline)
- ○

days months years numbers letters temperature height/weight other_____

Please use the reverse side of this questionnaire to draw your spatial pattern(s).

2B. If you indicated any association in section 2A (e.g., letters-to-colours, sequences in space) please tick to tell us whether you recall having these experiences during your childhood. If some associations started in childhood and some started only later in life, please tick the 'mixed' box.

- o Yes (I've had all of them since childhood/early adolescence)
- o No (they all started later in life) At what age?_____
- o Mixed* (some started in childhood, some in adulthood; please use the space below to let us know which associations started at which age, as best as you are able.)

_____
_____
_____
_____

***Please check that you have included ALL the associations you specified in section A. You can use the reverse if you need more room because we would like to understand when each one began.

2c. Please tick whether you know of any family members who have similar experiences to those described in section 2A above.

- o No
- o Yes Relationship to you:_____
  Types of experiences they have: _____

2d. Since the onset of your multiple sclerosis have you experienced any sensory disturbances?

- o No
- o Yes, visual disturbances. Please describe: _____
- o Yes, taste disturbances. Please describe: _____
- o Yes, hearing disturbances. Please describe: _____
- o Yes, smell disturbances. Please describe: _____
- o Yes, touch/tactile disturbances. Please describe: _____

Please use the space below if you would like to add further details about sensory disturbances or differences you have experienced: _____
_____
_____
_____
_____

Please tell us how frequently you have these sensory disturbances by writing below which one(s) you have for each time-frame (e.g., Daily: *visual disturbances and auditory disturbances*)?

- o Daily: _____
- o Weekly: _____
- o Monthly: _____
- o Very infrequently (less than one per month): _____
- o

2e. Please give any additional comments you think could be relevant. Thank you.

Appendix D

The genetics and inheritance of synesthesia

Chapter 2

The genetics and inheritance of synaesthesia

Julian E. Asher and Duncan A. Carmichael

Early evidence for genetic transmission of synaesthesia: prevalence, sex bias, and familiality

Synaesthesia is a neurodevelopmental condition characterized by anomalous sensory perception and associated alterations in cognitive function due to interference from synaesthetic percepts. For synaesthetes, a stimulus ('inducer') in one modality triggers an automatic, consistent sensation ('concurrent') in another modality. For example, in auditory-visual synaesthesia, sound triggers the perception of colour (Baron-Cohen, Wyke, and Binnie 1987; Ward, Huckstep, and Tsakanikos 2006). For many synaesthetes, the synaesthetic experience crosses modalities (e.g. sound-to-colour) while for other synaesthetes, the inducer and synaesthetic concurrent occur in different facets of the same modality; in visual grapheme-colour synaesthesia, for example, reading black-on-white text triggers the perception of colour (Mattingley et al. 2001; Ramachandran and Hubbard 2001). While synaesthetic inducers vary widely (e.g. sounds, tastes, graphemes), the resulting percepts are almost always visual, most often in the form of

colour although visually salient texture and movement have also been described (Asher et al. 2006; Baron-Cohen, Wyke, and Binnie 987; Simner et al. 2006).

The focus of this chapter is on the genetics of synaesthesia, which has important implications for its underlying aetiology. This aetiology can also be illuminated by examining the co-occurrence of synaesthesia with other cognitive phenotypes. Using neuroimaging techniques, alterations in white matter have been observed which could indicate the existence of overall increased connectivity in the brains of synaesthetes (Rouw and Scholte 2007). Studies also show that anomalous activation occurs in brain regions involved in colour processing when colour-experiencing synaesthetes are exposed to synaesthetic triggers (e.g. Nunn et al. 2002). Although many synaesthetes report their synaesthesia to be neutral or even pleasant, a growing body of evidence indicates that the simultaneous perception of normal and synaesthetic percepts can result in perceptual and cognitive dysfunction, with particularly strong effects on linguistic (Mattingley et al. 2001) and numerical (Green and Goswami, 2007) processing. Conversely, synaesthesia has also been implicated in 'positive' cognitive variation, including superior performance on certain perceptual tests (Ramachandran and Hubbard 2001; see Kim and Blake, Chapter 15, this volume) and improved recall (Smilek et al. 2002; see Meier and Rothen, Chapter 35, this volume). One of the most interesting connections is between synaesthesia and different forms of eidetic ('photographic') memory (Glicksohn, Steinbach, and Elimalach-Malmilyan 1999; Luria 1968), including absolute ('perfect') musical pitch (Rizzo and Eslinger 1989), and with heightened visual memory and imagery more generally (see Price, Chapter 37, this volume). Synaesthesia has also been implicated in cases of savantism (Baron-Cohen et al. 2007; Simner, Mayo,

and Spiller e. 009; see Spiller and Jansari, Chapter 36, this volume). We shall return later to examine these findings in our discussion of candidate genes.

The tendency of synaesthesia to cluster in families was first reported in 1883 by Sir Francis Galton (Galton, 1883), 71 years after synaesthesia was first described in the scientific literature (by Sachs in 1812; see Jewanski, Day, and Ward 2009). Despite this early observation, the genetics of synaesthesia remained unexplored for most of the twentieth century. During the early part of the last century, the techniques required to investigate the role of genes were simply unavailable; then later, following the development of genetic techniques, scientific resources were initially focused on identifying genes associated with more pathological conditions. Towards the very end of the twentieth century, however, the first attempts were made to investigate the hereditary nature of synaesthesia.

The development of suitable diagnostic methodologies to verify genuine synaesthetes played a key role in enabling researchers to investigate the genetics of synaesthesia. These methodologies relied (and still rely to this day) on the consistency of synaesthetes' reports about their percepts for any given trigger. Consistency is considered to be a hallmark of authenticity because for synaesthetes, the particular concurrent triggered by a given inducer (e.g. the very specific shade of red triggered by the letter 'A') tends to stay the same over time—even over very long time periods such as years and decades (e.g. Simner and Logie, 2008; see Johnson, Allison, and Baron-Cohen, Chapter 1, this volume, for review). In contrast, non-synaesthetes who invent analogous mappings by free association tend to be far less consistent over time, and this difference forms the basis of what has become known as the Test of Genuineness (TOG; Asher et al.

2006; Baron-Cohen, Wyke, and Binnie 1987; Baron-Cohen et al. 1993). The use of the TOG (or later versions; e.g. the Revised Test of Genuineness (TOG-R); Asher et al. 2006) to identify synaesthetes remains central to the study of synaesthesia in general, and to studies of its familiality.

At the birth of modern synaesthesia research in the final decades of the twentieth century, there was little empirical evidence regarding either the prevalence of synaesthesia or the ratio at which it occurs across the sexes, both of which are important for investigations of inheritance. Using the TOG, studies aimed to establish the prevalence and gender ratio as a starting point to explore the underlying genetics of the phenomenon. However, subsequent estimates of the prevalence of synaesthesia varied rather widely. Studies estimating prevalence by self-referral methods (i.e. asking synaesthetes to come forward through advertising media) estimated its prevalence at 0.05% to 1% of the population (Barnett et al. 2008; Baron-Cohen et al. 1996; Simner et al. 2006), while studies directly testing samples of the population without relying on synaesthetes' self-motivation found it to be as high as 4% (Simner et al. 2006; see Johnson Allison, and Baron-Cohen, Chapter 1, this volume, for an evaluation of previous prevalence methodologies). The use of different definitions of 'synaesthesia' for the various prevalence studies further complicated matters; many of the earlier studies (e.g. Baron-Cohen et al. 1996) focused on what was at the time the most well-known phenotype (auditory-visual synaesthesia, including music-colour synaesthesia which is now known to be a relatively uncommon phenotype) while the later studies included a much wider range of phenotypes (notably the most common variant, day-colour synaesthesia; e.g. Simner et al. 2006). Given recent evidence of clustering amongst

synaesthetic phenotypes and, specifically, the evidence that that auditory-visual and day-colour synaesthesia may be entirely separate phenomena (Novich, Cheng, and Eagleman 2011), revisiting prevalence figures for different clusters of synaesthesia would now be warranted. However, while the prevalence of synaesthesia has been controversial (for review, see Johnson, Allison, and Baron-Cohen, Chapter 1, this volume) the results of modern familiality studies provided clear evidence for a strong genetic component in synaesthesia by showing greater than 40% prevalence amongst the first- or second-degree relatives of known synaesthetes (Barnett et al. 2008; Baron-Cohen et al. 1996; Ward and Simner, 2005).[1]

Initial pedigree analyses of multiplex families (families containing multiple synaesthetes; Bailey and Johnson 1997; Baron-Cohen et al. 1996) supported the theory that synaesthesia may be a highly penetrant Mendelian disorder showing a major gene effect and a dominant mode of inheritance. By way of background, we provide here a brief overview of this terminology and then describe how such a theory was informed by early data on the relative proportion of female to male synaesthetes. Simply put, a

[1] The extent to which synaesthesia occurs in multiple family members may not be obvious because the condition can appear in different manifestations within the same family (e.g. a sister with coloured words may have a brother whose words trigger taste; e.g. Ward and Simner 2003). Indeed, data from a large-scale familiality study performed in Ireland with systematic screening of the first-degree relatives of synaesthetic probands revealed 11% of 'non-synaesthete' relatives to be synaesthetes of a different phenotype than the probands (Barnett et al. 2008). This means that reports from probands may underestimate the extent of familiality in synaesthesia if they focus on one variant only.

Mendelian disorder is a condition caused by a mutation in a single gene. Penetrance refers to the proportion of people possessing a particular variant of the gene in question who exhibit the phenotype (if the variant has a penetrance of 35%, only 35% of the people with that variant will show the phenotype). A condition is considered highly penetrant if a majority of people with that variant express the associated phenotype. To understand how these relate to inheritance and sex biases, we must also understand the inheritance of chromosomes.

Chromosomes are strings of nucleic acids and protein which carry genetic information in the form of genes. Humans possess 23 pairs of chromosomes, and inherit one chromosome per pair from each parent (for more information on this inheritance, see later in this chapter). As a consequence, we possess two copies (known as *alleles*) of the same gene, one on each chromosome—one inherited from our father, and one from our mother. In Mendelian terms, an allele can be *dominant* or *recessive*. If the possession of a single allele is sufficient to cause the expression of a trait in the phenotype, that allele is dominant. A condition with a dominant mode of inheritance only requires one of the two alleles in a particular pair to cause the phenotype to be expressed, so in effect, only one parent needs to carry the allele and pass it on to their offspring. If a pair of alleles consists of a dominant and a recessive copy, the dominant allele will mask the effect of the recessive allele in the phenotype. So for conditions that have a recessive mode of inheritance, the offspring must inherit a recessive copy from both parents before its effect can be expressed in the resulting phenotype.

The inheritance of synaesthesia initially appeared to be linked to the X chromosome because more females seemed to be affected than males and there was a

notable absence of confirmed cases of father-to-son (i.e. male-to-male) transmission. Females inherit two X chromosomes, while males inherit only one, this coming from their mother. Early studies suggested that there were six times more female synaesthetes than male synaesthetes (Baron-Cohen et al. 1996), and an initial meta-analysis performed by Bailey and Johnson in 1997 concluded that the available data at that time best fitted an X-linked dominant mode of inheritance. Because X-linked dominant inheritance alone cannot explain a female:male ratio greater than 3:1, the heavily skewed female presence was accounted for by suggesting that the putative 'synaesthesia gene' may be lethal in males causing death *in utero* (Bailey and Johnson 1997; Baron-Cohen et al. 1996). This could occur because males are hemizygous (meaning they possess only one copy of the X chromosome) and if that single copy were defective it could result in lethality.

However, more recent prevalence studies using different methodologies, and meta-analyses with larger data sets (e.g. Simner et al. 2006; Ward and Simner 2005) indicate that the female:male ratio may be far lower than previously believed (perhaps as low as 2:1 or even 1:1), calling the X-linked mode of inheritance into question. Furthermore, Ward and Simner (2005) showed that the male/female make-up of synaesthetes within families does not support a model of male lethality: female synaesthetes are just as likely to give birth to a son as to a daughter, and there was no difference in their number of brothers/sisters, aunts/uncles, or female/male cousins (see also Barnett et al. 2008). It was also suggested that the mode of inheritance was likely to be more complex, with skipped generations indicating reduced penetrance (Barnett et al. 2008; Simner et al. 2006; Ward and Simner 2005). Nonetheless, the enduring possibility of an elevated number of female synaesthetes in sex comparisons (if not 1:1, then

anywhere between 2:1 and 6:1 depending on methodology; and we return to this later) and the historical failure to detect any confirmed cases of male-to-male transmission made it imperative to directly test for the involvement of the X chromosome.

At present, the search for genes underpinning synaesthesia is still in its infancy. Empirical evidence linking specific areas of the genome to synaesthesia has been limited to a small handful of recent papers. Since the relative paucity of empirical studies precludes an extensive literature review, this chapter will focus on an in-depth discussion of the current literature, with particular emphasis on the seminal paper by Asher et al. (2009), the first genome-wide scan examining the genetic basis of synaesthesia. We begin with an overview of the genetic concepts that are relevant to the findings reviewed in these family linkage studies.

What is 'linkage' and why is it important?

The initial phase of sexual reproduction involves a stage called *meiosis*, which takes place in the ovaries and testes. The cells produced by meiosis are gametes (eggs or sperm). In meiosis, 23 pairs of chromosomes—half having originated from each parent—exchange fragments of DNA and are recombined. This results in the formation of a new and unique set of chromosomes containing genes from both parents. Unlike normal cells, gametes have only half the usual number of chromosomes: only 23, rather than 46. (When sexual intercourse subsequently occurs, these combine with the gamete chromosomes from the sexual partner, and genes from both parties are then inherited by the resulting offspring. In this way, genes from all four grandparents are inherited by the child.) In meiosis, the proximity of genes to each other on a given chromosome governs the likelihood of whether those genes become separated or remain together during the

process of recombination. The closer together genes are located, the more likely they are to remain together on the newly formed chromosome. This likelihood of being inherited together is referred to as ‘genetic linkage’.

In genetic studies, linkage is commonly measured as a ‘log of odds’ (LOD) ratio, which gives a statistical measure of the likelihood of genes remaining adjacent on the newly formed chromosome.[2] For example, a LOD score of 2 would indicate odds of 100:1 that linkage did not occur by chance and a LOD score of 3 would indicate odds of 1000:1. Linkage can be used to identify particular locations on a chromosome which contain genes responsible for a particular phenotype. *Microsatellites*, short sequences of repeating base-pairs of DNA distributed throughout the genome, can be used as genetic markers in familial linkage studies. In the same way that different family members can possess different versions of certain gene alleles, versions of microsatellites will also vary per family member. If we compare the microsatellites of affected versus non-affected family members, we can see which microsatellites are different and importantly, on

[2] As in any statistical analysis, it is necessary to ensure that the results obtained in a linkage study are not due to chance and the logarithm of the odds (LOD) score, introduced by Morton (1955), is a statistical measure of the evidence for linkage. LOD scores are calculated by comparing the likelihood of two alternative hypotheses: the likelihood of observing the genotypes obtained from the study at each meiosis under the hypothesis of linkage, to the likelihood of observing that genotype under the null hypothesis of no linkage, in a likelihood ratio. If the likelihood ratio (‘odds’) is greater than 1 it is said to support linkage. This is normally expressed as Z, the LOD score. The probability of linkage in a set of families can be calculated by summing the LOD scores across the sample.

which chromosomes these differences appear. Because of the principles governing genetic linkage, we can deduce that the locations at which the microsatellites differ are also likely to be the locations at which the genes causing the phenotype under investigation (e.g. synaesthesia) are found. The implication is that the genes responsible for producing the phenotype will be in close proximity to the particular microsatellite markers found in affected family members.

Family-based linkage studies

Evidence of linkage to chromosomes 2, 5, 6, and 12 (2q24, 5q33, 6p12, and 12p12)

Asher et al. (2009) conducted the first whole-genome scan for susceptibility genes linked to synaesthesia. This study was conducted in a sample of 196 individuals who experience colour synaesthesia triggered by sounds and/or by spoken words (referred to henceforth as 'auditory-visual synaesthesia') from 43 multiplex families. Families were recruited in two waves, with 19 families recruited during the initial recruitment period (Wave 1), and 24 families recruited during the second recruitment period (Wave 2). Of particular interest, the sample included two families reporting male-to-male transmission of synaesthesia (see Figure 2.1) and we return to this later in the chapter. Before starting the genome scan, Asher et al. confirmed the power of this sample to detect a major gene effect via computer simulations (SLINK; Weeks, Ott, and Lathrop, 1990) using the best available data on prevalence and mode of inheritance.

The sample was phenotyped using the TOG or TOG-R (Asher et al. 2006; Baron-Cohen et al. 1996). Phenotyping revealed 121 affected individuals and 68 unaffected

individuals; seven individuals were treated as phenotype unknown (five children who were too young to undergo diagnostic testing and two reported synaesthetes who were not available for testing). Genomic DNA was extracted from blood samples (Wave 1) or buccal swabs (Wave 2). Following extraction, DNA samples underwent whole-genome amplification—the production of larger usable samples of DNA from minute quantities.

Four hundred and ten highly polymorphic microsatellite markers were used in the genome scan. Asher et al. conducted a multipoint non-parametric linkage (NPL) analysis which estimates allele-sharing among all affected family members. NPL analysis makes no assumptions about the mode of inheritance, which makes it more sensitive to linkage than parametric analysis (where a mode of inheritance must be specified) but it is less powerful as the candidate regions identified tend to be larger. The analysis detected 15 potential candidate regions with a LOD score >1 on 11 chromosomes (see Figure 2.2). Single-point NPL LOD scores for the linkage peaks were calculated using the same set of parameters. These regions were then fine mapped at higher density with additional microsatellites. Further NPL analysis of the fine-mapped regions identified four candidate regions with LOD >2. Suggestive linkage under the Lander and Kruglyak criteria (LOD >2.2; Lander and Kruglyak 1995) was detected at two of these regions, on chromosomes 5q33 (LOD = 2.3, $p = 0.0006$) and 6p12.3 (LOD = 2.37, $p = 0.0005$). All regions with LOD scores >2 were supported by single-point LOD scores >1 from a minimum of three markers.

In addition, Asher et al conducted a multipoint heterogeneity LOD (HLOD) score analysis using models derived from the best available estimates following pedigree analysis. HLOD analysis has greater power to detect linkage in the context of substantial

genetic heterogeneity than either traditional parametric or NPL analyses. Given the lack of consensus regarding the prevalence of auditory-visual synaesthesia in published prevalence studies (Baron-Cohen et al. 1996; Simner et al. 2006) a conservative estimate (genotype frequency = 0.01) was used for disease allele frequency in the analysis. Pedigree analysis of the sample revealed a dominant inheritance pattern (see Figure 2.3); but because skipping of generations has been observed both in this sample and in previous prevalence studies, initial penetrance for the dominant model was set at a variety of levels (see Asher et al. 2009 for details). Three models provided the best fit: dominant (penetrance = 0.65), dominant (penetrance = 0.90), and recessive (penetrance = 0.75). HLOD analysis was conducted using fine-mapping data whenever this was available and data from the primary genome scan data for the remainder of the genome.

Genome-wide empirical *p*-values for the HLOD analysis were calculated, correcting for multiple testing by estimating the null distribution over all genetic models in each simulation. Under the null hypothesis of no linkage anywhere in the genome, 1000 replicates were generated, retaining original pedigree structures and missing data patterns in order to reflect the real data. In accordance with the Lander and Kruglyak recommendation, thresholds were calculated for suggestive linkage (1000 LOD scores of equal or greater size in 1000 simulations), significant linkage (50 LOD scores of equal or greater size in 1000 simulations), and highly significant linkage (1 LOD score of equal or greater size observed in 1000 simulations) across the entire marker set. These simulations generated the following empirical genome-wide significance thresholds: 2.03 for suggestive linkage, 2.97 for significant linkage (p <0.05), and 5.70 for highly significant linkage (p <0.001). The empirical *p*-value for a peak in the analysis was set by counting

how often a simulated unlinked LOD score of greater or equal value is seen in 1000 simulations.

The HLOD analysis detected one region of significant linkage on chromosome 2q24.1 (HLOD = 3.025, empirical genome-wide $p = 0.047$) and three regions of suggestive linkage on chromosomes 6p12.3 (HLOD = 2.272, empirical genome-wide $p = 0.275$), 12p12.1 (HLOD = 2.849, empirical $p = 0.073$), and 9q33.1 (HLOD = 2.473, empirical $p = 0.188$). A total of 23 regions with HLOD >1 were detected, 12 of which were also detected by the NPL analysis (including the suggestive linkage peak on chromosome 6). The linkage results on chromosomes 2, 6, and 12 were supported by single-point HLOD scores based on the same parameters. The result on chromosome 9 was only supported by a single marker in the single-point analysis, indicating a possible statistical artefact, and hence this locus was not considered a candidate region. Additional support for the HLOD results came from an additional set of NPL analyses focusing on the subsets of families contributing to each HLOD peak (see Figure 2.4). This analysis reveals a significant LOD score increase over the initial NPL analysis, indicating that the presence of significant heterogeneity at these loci was likely to have diminished the linkage signal in these regions and providing strong support for the HLOD result.

In sum, Asher et al.'s pioneering genome-wide scan for susceptibility genes for auditory-visual synaesthesia identified four candidate regions, with significant linkage to chromosome 2q and suggestive linkage to chromosomes 5q, 6p, and 12p under the criteria proposed by Lander and Kruglyak (1995). Notably, the results failed to support previous suggestions of linkage to the X chromosome, and we discuss this in further detail later. While the resolution of this genome scan makes identifying potential

candidate genes within the candidate regions challenging, the uncertainty about the aetiology of synaesthesia makes the genetic locations of these regions of particular interest. We review this next, following Asher et al. (2009).

The marker with the highest LOD score in the region of significant linkage on chromosome 2q (D2S142, with HLOD = 3.025) has previously been linked to autism (IMGSAC 2001). This is particularly interesting in light of the fact that sensory abnormalities are a significant feature of autism-spectrum disorders (ASD; Ashwin et al. 2009; Belmonte et al. 2004), with synaesthesia itself sometimes reported as a symptom (Harrison and Hare 2004). Moreover, a recent study at the University of Cambridge indicates a possible increased prevalence of synaesthesia among people with ASD (S. Baron-Cohen, personal communication). Furthermore, neuroimaging studies have revealed that auditory stimuli trigger responses in both auditory and visual brain regions in both autistic individuals (Kemner et al. 1995) and auditory-visual synaesthetes (Nunn et al. 2002). Studies have also shown increased neural connectivity in the brains of people with ASD (Courchesne, t al. 2005), which is particularly notable given recent evidence for increased connectivity in the brains of synaesthetes (Rouw and Scholte 2007; see Rouw, Chapter 25, this volume). Finally, recent studies also suggest that savantism, long connected with ASD, may in some cases result from the combination of ASD and synaesthesia together (Baron-Cohen et al. 2007; Simner, Mayo, and Spiller e. 009; see Spiller and Jansari, Chapter 36, this volume).

There are a number of interesting candidate genes on chromosome 2. *TBR1* (MIM[3] 604616) induces the transcription of genes regulated by the T-box element that are thought to be integral in embryo development (Bulfone et al. 1995; Hsueh et al. 2000; Smith 1999). These genes include reelin (*RELN* (MIM 600514)), which is central in the development of the cerebral cortex; the brains of *tbr1* knockout mice develop abnormalities in laminar cortical organization, which could in theory play a role in the altered neural connectivity of synaesthetes (Hevner, Miyashita-Lin, and Rubenstein 2002; Hevner et al. 2001). Another set of genes in this region, the sodium channel alpha subunit genes *SCN1A* (MIM 182389) and *SCN2A* (MIM 182390) encode voltage-gated sodium channels throughout the central nervous system (Whitaker et al. 2000, 2001). Defects in these genes have been linked to seizures (MIM 607745) (*SCN2A*) (Berkovic et al. 2004; Herlenius et al. 2007) and epilepsy (MIM 604233) (*SCN1A*) (Escayg et al. 2000; Wallace et al. 2001). This has implications for synaesthesia since epilepsy has been traced to alterations in the excitability and connectivity of neural networks (McCormick and Contreras 2001) and the 'disinhibition' theory of synaesthesia posits lowered excitability thresholds leading to increased cross-talk between neurons. Finally, rare mutations of *TBR1*, *SCN2A*, and a nearby gene *SCN3A* have again been also observed in autism (Bacchelli et al. 2003; Weiss et al. 2003). Additional noteworthy candidate genes in this region also include *ERMN* (MIM 610072), an analogue of which is upregulated

[3] Information on specific genes can be found online in the Mendelian Inheritance in Man (MIM) database (<http://www.ncbi.nlm.nih.gov/omim>); the MIM numbers used here refer to the unique identifier each gene has been assigned in this database.

during the period of active myelination of central nervous system axons in rats (Brockschnieder, t al. 2006).

The region on chromosome 5q detected by the Asher et al analysis includes *DPYSL3* (MIM 601168). This gene plays a role in axonal growth and guidance, and in neuronal differentiation (Quinn, Gray, and Hockfield 1999). The fact that *DPYSL3* is highly expressed in the very early stages of development but not in the adult brain (Choi et al. 2005) makes it of potential interest in the context of previous hypotheses for 'neonatal synaesthesia' (Maurer 1993). In the 'neonatal synaesthesia' theory (see Maurer, Gibson, and Spector, Chapter 3, this volume) all babies are assumed to experience sensory input in an undifferentiated way. While this experience disappears over the course of normal development in most people, synaesthetes may retain some of these neonatal connections. The role of *DPYSL3* in neuronal differentiation and neural architecture more generally is therefore compelling given the known alternations in neural architecture in synaesthetes.

The region on chromosome 6 detected in Asher et al.'s analyses has been strongly linked to dyslexia (*DYX2*) and is specifically associated with problems in phonological and orthographic processing (Fisher et al. 2002; Kaplan et al. 2002). Candidate genes for dyslexia include two genes in this region, *KIAA0319* (MIM 609269) (Paracchini et al. 2006) and *DCDC2* (MIM 605755) (Meng et al. 2005), which both play a role in neuronal migration. This has interesting implications for synaesthesia given the altered neural architecture implicated in the disorder. It is also notable given that several of the most common synaesthetic phenotypes are triggered by language units, including the two most common forms of synaesthesia (day-colour synaesthesia and grapheme-colour

synaesthesia; Simner et al. 2006). It is also interesting given early clinical reports of an elevated prevalence of dyslexia among synaesthetes (S. Baron-Cohen, personal communication) although this is complicated by reports of *superior* orthographic abilities in other synaesthetes (Linn et al. 2008). Finally, the region on chromosome 6 detected in our analyses has also been linked to juvenile myoclonic epilepsy (MIM 606904), and a causative gene involved in apoptosis has been found (*EFHC1* (MIM 608815); Suzuki et al. 2004). In the neonatal theory of synaesthesia described earlier, the perseverance of early undifferentiated sensory experience into adulthood has been attributed to a possible failure in apoptosis (the normal process of cell death by which early hyper-connectivity becomes 'pruned' in development; see Maurer, Gibson, and Spector, Chapter 3, and Mitchell, Chapter 27, this volume). The mutation of these epilepsy-linked genes in chromosome 6 seen in epileptic families lowers the gene's apoptotic effect causing problems in neuronal pruning in development; a similar effect in synaesthetes could contribute to the retention of early synaesthetic pathways.

The region identified on chromosome 12 contains *GRIN2B* (MIM 138252), the N-methyl-D-aspartate (NMDA) receptor 2B subunit gene. NMDA receptors may play a central role in long-term potentiation and the consolidation of learning and memory (Shimizu et al. 2000), a finding of particular interest in light of studies which have shown a connection between synaesthesia and improve recall. Over-expression of this gene in mice has resulted in enhanced learning and memory (Tang et al. 1999). *GRIN2B* has been further linked to autism (McCauley et al. 2005), which is particularly notable given our earlier discussions of improved memory, savantism, and autism in synaesthesia.

Evidence of linkage to chromosome 16 (16q12.2–23.1)

Further research into the genetic basis of synaesthesia has highlighted a region of interest on chromosome 16. Tomson et al. (2011) conducted a family-based linkage study investigating a different form of synaesthesia, a phenotype they called *coloured sequence synaesthesia* in which linguistic inducers that fall in sequences (e.g. days, letters, numbers, months) trigger the perception of colour. There is some potential phenotypic overlap with the Asher et al. (2009) study (e.g. synaesthetes with coloured letters; these often experience colour for spoken words—although spoken words can also be triggered by phonemes). Testing five families (n = 48), Tomson et al. discovered linkage to a region of chromosome 16 (16q12.2–23.1) in two of the five families. Within this region, six candidate genes were selected for further analysis on the basis of having functions that may play a role in the development of abnormal neural connectivity between regions of the cortex. However, further sequencing of these genes did not reveal any evidence to suggest that they contributed to the development of synaesthesia in this subgroup. While these results must be interpreted cautiously given the small size of the sample and hence the lack of statistical power, this result is certainly of interest.

The fact that separate familial linkage studies investigating different phenotypes of synaesthesia identified linkage on distinct regions of different chromosomes raises a broader issue worthy of further discussion. Is there a single synaesthesia genotype that lends itself to a general predisposition to developing synaesthesia, with differences in sensory input or other environmental factors contributing to the development of a particular phenotype? Or do specific genes contribute directly to the occurrence of particular synaesthesia phenotypes? It has been demonstrated that certain types of synaesthesias tend to co-occur more than others (Simner et al. 2006) and synaesthesia

may be classified according to broader groupings (Novich, Cheng, and Eagleman 2011). Novich and colleagues considered the co-occurrence within individuals of 22 different types of synaesthesias in a group of 12,127 synaesthetes who had been verified using a type of TOG via the online interface at <http://www.synesthete.org> (Eagleman et al. 2007). Novich, Cheng, and Eagleman (2011) reported five distinct clusters of synaesthesia forms, in that a person possessing more than one type of synaesthesia is more likely to have types within the same cluster, rather than across clusters. No Celabelled these five clusters *coloured music synaesthesias*, *coloured sequence synaesthesias* (see earlier), *non-visual sequela synaesthesias* (colours triggered by physical touch or some emotional component), *spatial sequence synaesthesia* (where sequenced units are experienced as having spatial locations), and *coloured sensation synaesthesias* (whose inducers are non-visual; e.g. taste).

The findings of this study suggest that what we think of as 'synaesthesia' may in fact encompass several distinct phenomena each with their own patterns of expression. This has important implications for our hypotheses about the genetic roots of synaesthesia which are more likely, therefore, to be multifaceted. Specifically, Novich, Cheng, and Eagleman (2011) suggest that each group of synaesthesias may have independent underlying neural pathways, the development of which may be driven by distinct sets of genes. Importantly then, although there was likely to have been a partial overlap in the populations of synaesthetes sampled by Tomson et al. (2011) and by Asher et al. (2009), there was also a key difference, the importance of which is now magnified by the findings of Novich, Cheng, and Eagleman: Asher et al.'s sample included music-colour synaesthetes, while Tomson et al.'s sample were solely triggered by language

sequences. The findings by Novich and colleagues tell us that these may represent qualitatively and meaningfully different forms (in the terminology of Novich and colleagues, *coloured music synaesthesia* and *coloured sequence synaesthesia*), each with their own genetic mechanism. While further empirical evidence will be required before firm conclusions can be drawn, it is clear that the relationship between genotype and phenotype is unlikely to be straightforward.

Why doesn't X mark the spot?

We end our chapter with a re-evaluation of what was perhaps the most important early hypothesis about the genetic roots of synaesthesia. Early studies had suggested that there may be a major locus on the X chromosome, due to an absence of confirmed male-to-male transmission and a female:male ratio of up to 6:1. However, neither Asher et al. (2009) nor Tomson et al. (2011) found evidence for a major locus on the X chromosome and so these findings indicate a need to revisit our early understanding of the mode of inheritance of synaesthesia.

The early assumption about an absence of male-to-male transmission has been subsequently refuted by the verification of two cases of male-to-male transmission of synaesthesia in the study by Asher et al. (2009). While it is possible that these two families may carry a very rare autosomal mutation and that the majority of cases of synaesthesia are in fact X-linked, the results of the genome scan indicate that this is unlikely to be the case. It is also possible that the marker density was insufficient to detect significant linkage to a locus on the X chromosome; increased marker density (e.g. with an approach known as single-nucleotide polymorphism (SNP)-based high-density genotyping) would indeed increase the amount of information obtained from the

chromosome (Sawcer et al. 2004). However, studies directly comparing the results of microsatellite and SNP-based genome scans indicate that while the increased information content may raise unremarkable LOD scores to the level of suggestive linkage, or suggestive scores to the level of genome-wide significance, it has not resulted in the detection of significant linkage in areas where the microsatellite scan failed to do so (Middleton et al. 2004; Sawcer et al. 2005). This suggests that increased marker density would be unlikely to reveal a major locus on the X chromosome, though it might reveal stronger linkage in the region where the maximum LOD score was obtained.

In the absence of evidence for a major locus on the X chromosome, an alternate explanation for earlier observed high female:male ratios must be found. We noted earlier that is possible that the female predominance observed in previous studies of synaesthesia does not in fact reflect the true ratio among affected persons (Simner et al. 2006; Ward and Simner 2005). Simner and colleagues have suggested that an especially high female skew may emerge when synaesthetes are asked to self-refer for study (e.g. Baron-Cohen et al. 1996), since women have been shown to be more likely to come forward to report atypical behaviour (Dindia and Allen 1992). Furthermore, when prevalence was assessed without the potential for self-referral, there was a non-significant difference across the sexes (Simner et al. 2006). However, the complete absence of female predominance seems highly unlikely given the consistency of a small but persistent female bias across multiple studies (Simner et al. 2009). Simner and colleagues have pointed out a repeated trend in the direction of a female bias of approximately 2:1 (e.g. Simner et al. 2006, 2009) and suggest that poor power alone in previous statistical tests makes it possible that a female bias (perhaps of around 2:1) may

re-emerge in larger sample testing.[4] Furthermore, referral bias usually accounts for only a slight variation (10%) in response rates (Dindia and Allen 1992) again making it likely that high early estimates were at least pointing in the right direction.

Assuming a slight but significant female bias may yet be found in future large-scale testing, a number of genetic factors could account for this. Synaesthesia may be a complex trait involving multiple genes with relatively small individual genetic effect, or it may be subject to multiple modes of inheritance or locus heterogeneity. If synaesthesia is an oligogenic or complex trait subject to a threshold of liability, the families with father-to-son transmission may represent cases in which threshold was reached without

[4] Studies showing an apparent female bias without self-referral have not, thus far, been entirely free of the potential for this confound. Barnett et al. (2008) report a female bias of approximately 6:1 in a study that looked at both self-referred probands, and their family members who were directly contacted by the researchers. However, Barnett and colleagues were only able to objectively assess all first-degree family members for 17 out of 53 probands, which meant that 81 of their 92 synaesthetes were either objectively unconfirmed cases or self-referred (J. Simner, personal communication). A similarly high female bias of approximately 4:1 (based on the members of n = 5 families) reported in Tomson et al. (2011) has now been corrected by erratum to 2.7:1. This brings it in line with an earlier study by Ward and Simner (2005) who showed a female:male ratio of 2:1 when they also attempted to eliminate a referral bias by looking within the families of synaesthetes (n = 85 families). However, Simner and colleagues point out that this type of ratio may yet be an overestimate from self-referral, since families with a lone synaesthete are less likely to come to the attention of researchers if that synaesthete is male (Simner et al. 2006).

any contributions from mutations on the X chromosome, whereas the other families represent cases in which a mutation or mutations on the X chromosome plays a role in reaching threshold. Greater prevalence of the X chromosome mutation(s) would account for the X-linked transmission pattern observed in the majority of synaesthetic families. Additionally, the threshold may differ between the sexes, with one sex requiring fewer disease-causing factors to display the phenotype (Carter 1976). In the context of a complex disorder, the findings on the X-chromosome could reflect the existence of a locus with limited effect. Finally, a specific hypothesis has been proposed by Mitchell (Chapter 27, this volume) who points to a priori sex differences in connectivity, with women's brains showing greater overall connectivity across the entire brain. Mitchell suggests that a condition such as synaesthesia, which results from atypical hyper-connectivity may certainly be expected to be greater in females than males.

Summary and future directions

The findings reviewed here have important implications for our overall understanding of synaesthesia, as they indicate that its genetic basis is rather more complex than originally believed. Separate linkage studies (Asher et al. 2009; Tomson et al. 2011) conclude that rather than being caused by a single gene at a single locus with a single mode of inheritance, synaesthesia is more likely to arise from the effects of multiple genes at multiple loci with different modes of inheritance. The discovery of multiple peaks with relatively small genetic effects combined with the detection of significant linkage on chromosome 2q and suggestive linkage to chromosomes 5q, 6p, and 12p (Asher et al. 2009) is consistent with a complex disorder with considerable genetic heterogeneity. This conclusion is further reinforced by the discovery of linkage to a region on chromosome

16, found in participants exhibiting a different, yet possibly overlapping, phenotype (Tomson et al. 2011).This could potentially mean that there are a number of causative loci, with different loci producing the clinical phenotype in particular families. This would be consistent with findings in other neurodevelopmental disorders such as specific language impairment (MIM 606711) (Newbury, Bishop, and Monaco 2005) and dyslexia (MIM 127700) (Paracchini, Scerri, and Monaco 2007) where both gene–environment and gene–gene (epistatic) interactions are believed to play an important role.

Given the evidence for substantial genetic heterogeneity, it is likely that the development of meaningful *endophenotypes* will play a key role in future research. A genetic endophenotype is a well-defined, quantifiable measure describing one subdimension of a complex disease phenotype. Increasing phenotypic homogeneity through the use of endophenotypes has been shown to increase the power to find susceptibility genes (Buxbaum et al. 2004; Shao et al. 2002). It will be necessary to revisit the phenotypic data to identify subdomains of the synaesthetic phenotype that may serve as useful endophenotypes, ideally by providing quantitative measures. The amount of information generated by more recent tests of genuineness (e.g. the TOG-R; or the tests at <http://www.synesthete.org>) makes this test a logical candidate for use in endophenotyping. However, it is notable that while the use of endophenotypes can produce more statistically reliable data they do not necessarily have a simpler genetic architecture than the underlying disease, and that the genetic effect sizes observed with endophenotypes are not necessarily larger than those observed with the global disease phenotype (Flint and Munafo 2007); care will need to be taken to ensure adequate statistical power to detect genes of small effect.

Even with this additional data, it may not be possible to specify a single mode of inheritance for synaesthesia. The recent evidence from Novich and colleagues based on a large sample of synaesthetes has shown that types of synaesthesias can be clustered together in certain subtypes and different varieties of synaesthesias are more likely to co-occur in affected individuals if they belong to the same subgroup (Novich, Cheng, and Eagleman 2011). This conclusion raises the possibility that multiple genotypes may be responsible for the expression of distinct groups of different phenotypes. Indeed, we cannot currently say for certain whether Asher et al. (2009) and Tomson et al. (2011) have identified different loci that contribute some proportion of genetic effect to an overarching 'synaesthesia genotype' or whether they are loci central to quite distinct subgenotypes. For this reason, further investigation into the subtleties of the relationship between phenotypes should prove to be a fruitful line of inquiry. The use of additional phenotyping methods, notably neuroimaging, to define subphenotypes within the synaesthetic population with greater precision would provide important additional information and facilitate the search for susceptibility genes.

Even if most cases are due to oligogenic inheritance, it is possible (even probable given what has been seen in other neurodevelopmental disorders) that there are individual families (e.g. as in specific language impairment; Fisher et al. 1998) or subgroups of families (e.g. as in Tourette's syndrome (MIM 137580;)Abelson et al. 2005);and ASD (MIM 209850;)artin and Ledbetter 2007)) which show Mendelian inheritance of a mutation although genome scans indicate that the overall inheritance of the disease is genetically complex. While they are likely to be considerably rarer than those showing more complex inheritance, the detailed analysis of families with known chromosomal

abnormalities or detectable copy number variants may facilitate the identification of candidate genes and the delineation of pathways which play an important role in the aetiological process.

Scientific exploration of the role played by genes in human cognition has only recently begun. Formerly an obscure condition, synaesthesia has attracted growing interest for its potential to advance our understanding of both typical and atypical human cognition and perception. A greater understanding of the neural mechanisms underlying synaesthesia has important implications for other neurodevelopmental disorders, many of which (e.g. ASD (arrison and Hare 2004); Williams–-euren syndrome (MIM 194050; Levitin et al. 2005)) involve abnormal sensory perception. Moreover, as synaesthetic perception occurs in the absence of direct sensory stimulation, it may offer insight into how the human brain integrates sensory data into conscious perception, and may even illuminate the neural basis of consciousness (Gray et al. 2002; see Sagiv and Frith, Chapter 45, this volume). The eventual identification and functional characterization of susceptibility genes linked to synaesthesia will yield fundamental insights into the role of genetics in human cognition and perception.

Acknowledgements

DAC was supported in part by grants EP/F500385/1 and BB/F529254/1 for the University of Edinburgh, School of Informatics Doctoral Training Centre in Neuroinformatics and Computational Neuroscience (<http://www.anc.ed.ac.uk/dtc>) from the UK Engineering and Physical Sciences Research Council (EPSRC), UK Biotechnology and Biological Sciences Research Council (BBSRC), and the UK Medical Research Council (MRC).

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Figure 2.1

Pedigrees of families reporting male-to-male transmission of auditory-visual synaesthesia. Hatched = phenotype unknown. Asterisk indicates an individual from whom DNA was obtained.

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Figure 2.2

Results of the whole-genome scan. Generated by NPL analysis using MERLIN 1.1a with the exponential function.

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Figure 2.3

Representative sample of pedigrees. Hatched = phenotype unknown. Deceased individuals are included for information only with phenotypes based on clinical interview, and were excluded from the analysis.

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Linkage to Chromosomes 2q24, 5q33, 6p12, and 12p12, pp. 279–285, Copyright (2009), with permission from Elsevier.

Figure 2.4

Increase in NPL during subset analysis. Dashed black = NPL (full sample); blue = NPL (contributing families); dashed and dotted red = HLOD (chromosome 2 = dominant, 0.90; chromosome 6 = dominant, 0.65; chromosome 12 = recessive, 0.75). Contributing families are those showing linkage to the HLOD peak.

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Appendix E

The immune hypothesis of synesthesia

frontiers in
HUMAN NEUROSCIENCE

OPINION ARTICLE
published: 11 September 2013
doi: 10.3389/fnhum.2013.00563

The immune hypothesis of synesthesia

***Duncan A. Carmichael*[1,2,3]* and Julia Simner*[1]**

[1] Department of Psychology, University of Edinburgh, Edinburgh, UK
[2] Institute for Adaptive and Neural Computation, University of Edinburgh, Edinburgh, UK
[3] Division of Psychiatry, Royal Edinburgh Hospital, University of Edinburgh, Edinburgh, UK
*Correspondence: d.a.carmichael@ed.ac.uk

Edited by:
Nicolas Rothen, University of Sussex, UK

Reviewed by:
Berit Brogaard, University of Missouri, USA

Keywords: synesthesia, genetics, immunity, neurological basis, genes, cortical connectivity, immune system

Synesthesia is a hereditary, neurological condition in which a wide range of common stimuli (e.g., letters, sounds, flavors) trigger unexpected secondary sensations, for example, synesthetes listening to music might see colors in addition to hearing sounds (Ward et al., 2006; see Simner and Hubbard, 2013, for review). Current explanations of synesthesia posit structural and/or functional differences in the synesthete brains, and frame their models in terms of excess cortical connectivity or altered cortical feedback. Here, we propose an immune hypothesis of synesthesia, which supplements existing models by suggesting how such altered connectivity may arise and how associations between synesthesia and other conditions might be explained.

Two categories of model seek to explain the generation of synesthetic experiences, and more recent models are a hybrid of both (Brang et al., 2010). The *cross-activation model* (Ramachandran and Hubbard, 2001) suggests that excess connectivity between functional areas of the cortex allows activation in one cortical area (e.g., auditory cortex) to directly trigger activation in another (e.g., visual cortex). Evidence in support of this model comes for example from diffusion tensor imaging (DTI) and shows that excessive connectivity is indeed a feature of the synesthetic brain (Rouw and Scholte, 2007). *Re-entrant* and *disinhibited feedback models* propose that synesthetic sensations are caused by disinhibited feedback from higher cortical areas (e.g., in parietal lobe) failing to suppress non-relevant activation from lower cortical areas (Grossenbacher and Lovelace, 2001). This type of disinhibited feedback may result from excessive activity of excitatory neurons within the delicate balance between both excitatory and inhibitory neurons in the brain (Hubbard et al., 2011). Despite appearing superficially different, connectivity and feedback models need not be mutually exclusive. It is unlikely that altered feedback happens entirely in the absence of changes in cortical connectivity, given the Hebbian principle that simultaneous activity strengthens interconnectivity between neurons. Therefore, these two approaches might be considered somewhat unified in that connectivity models propose aberrant connectivity as the primary causal mechanism underlying synesthesia whereas feedback models might allow altered connectivity as an *indirect* consequence of disinhibited feedback.

While these models are now more than a decade old, explanations of *how* these cortical characteristics might arise have proven elusive thus far (but see Brang and Ramachandran, 2008; Mitchell, 2013) and we explore this here. Synesthesia is thought to be primarily neurodevelopmental in nature (Spector and Maurer, 2009). Consequentially, known processes of brain development are likely to be implicated in its emergence. We propose that insight might be gained from examining the functionality of genes that regulate the types of altered synesthetic cortical connectivity assumed in these models above (i.e., genes for axon guidance, synapse density). This is the approach we follow here.

DUAL GENE FUNCTIONALITY: CONNECTIVITY AND IMMUNITY

Above, we saw that current models link synesthesia to altered structural connectivity, misregulated feedback mechanisms, or a combination thereof. Explaining how synesthesia develops might therefore come from considering the developmental processes responsible for cortical connectivity. The immune system is known to play a significant role in these processes (for review, see Boulanger, 2009) and we ask here whether a propensity to develop synesthesia may be linked to the expression of immune proteins in the CNS, since this expression can be conferred by genes with functions of both immunity and cortical development. Indeed, many genes have been shown to have precisely this dual functionality (Boulanger, 2009). As well as their immunity function, such genes act in cortical development, altering structural and/or functional connectivity by influencing the development of axonal guidance, synaptic connectivity, and synaptic pruning. One outcome of these changes may therefore be the anomalous pattern of connectivity proposed by the cross-activation theory in the development of synesthesia. Alternatively, the immune system could have a direct influence on excitatory neuronal activity, leading to the outcomes proposed by disinhibited feedback models. This is because the immune system plays an important role in the initial development and subsequent plasticity of glutamatergic synapses, the primary excitatory transmission pathway in the mammalian cortex (Fourgeaud and Boulanger, 2010).

CAN THE IMMUNE SYSTEM INFLUENCE THE DEVELOPMENT OF THE BRAIN?

Is it plausible to make a link between the immune system and regulation of the central nervous system (CNS)? Isolated from the rest of the body by

the blood-brain-barrier, the CNS was once thought to barely interact with the immune system, leading to the long held view that the CNS was "immune privileged" (McAllister and van de Water, 2009). However, research now shows a complex communication between the CNS and immune system, with wide-reaching consequences for brain regulation and development, both in health and disease (Elmer and McAllister, 2012). Immune proteins are known to play a role at many stages in the developmental pathway. They are integral components of phases critical to brain development and plasticity, such as neuronal guidance, synapse development, and synaptic remodeling (Boulanger, 2009). We therefore hypothesize that CNS and immune system interaction may be the biological mechanism which confers the predisposition to develop synesthesia.

Which aspects of the immune system are known to exhibit the type of dual functionality under discussion in this article (i.e., functionality in both immunity and cortical development)? Several areas of this extraordinarily complicated system are worth highlighting. The *complement system* is one possible candidate—a complex cascade of protein interactions involved in immunity which has also been shown to play an important role in tagging of synapses to be eliminated by pruning during development (Stephan et al., 2012). Another candidate relates to cytokines, which are immune proteins that have also been shown to play significant roles in neurogenesis and synaptic plasticity (Bauer et al., 2007). A third candidate relates to major histocompatibility complex (MHC) proteins, which are an integral part of the adaptive immune system found on the surface of the majority of nucleated cells and widely expressed in neurons of the CNS (Boulanger, 2004). In addition to fulfilling a crucial function in immune response, MHC class I molecules and related components are thought to be involved in a range of developmental processes, such as activity-dependent plasticity and synaptic refinement (Boulanger, 2009). The MHC locus contains several hundred genes, and has also been widely implicated in a range of autoimmune conditions, such as multiple sclerosis (MS), irritable bowel syndrome (IBS), and rheumatoid arthritis (Fernando et al., 2008). We point out, however that the immune system consists of many hundreds of individual factors and processes. Given this, our suggestions above should be considered speculative and by no means exhaustive. Nevertheless, we consider them to each be plausible candidates for future investigation.

We end this section by asking whether existing studies into the genetics of synesthesia would support our immunity hypothesis. In other words, have they identified areas of the genome containing immune system genes? Research into synesthesia genetics is in its infancy and as yet, there are insufficient data to draw firm conclusions. No synesthesia genes have yet been identified and no firm mode of inheritance has yet been elucidated. However, evidence from the two existing studies on the genetics of synesthesia (Asher et al., 2009; Tomson et al., 2011) have identified several chromosomal regions of interest, and these regions do contain immune function genes. Asher et al. (2009) found significant linkage to chromosome 2q24 and possible linkage to areas on other chromosomes (5q33, 6p12, and 12p12), while Tomson et al. (2011) identified a candidate region on chromosome 16q12.2-23.1. The authors of both studies draw the conclusion that synesthesia is likely to be a condition influenced by a variety of genes in multiple loci. Nonetheless, the chromosomal regions of interest highlighted in these two investigations do contain immune function genes (e.g., interleukin-17, a cytokine protein found on chromosome 6p12), although we wish to be clear that many other viable candidates also lie outwith these regions.

THE IMMUNE HYPOTHESIS AS A FRAMEWORK FOR THE STUDY OF CO-MORBIDITIES

An immune hypothesis of synesthesia might additionally explain recent co-morbidity data which suggests that having synesthesia may be associated with increased risk of other clinical conditions. Carruthers et al. (2012) report an association between synesthesia and IBS, having found an elevated prevalence of synesthesia in a population of people with IBS. Other researchers have raised the possibility that synesthesia may also be found at elevated rates within populations with autism (Baron-Cohen et al., 2007) or migraine (Alstadhaug and Benjaminsen, 2010). The immune system plays a prominent role in all of these conditions (Collins, 2002; Bruno et al., 2007; Enstrom et al., 2009), suggesting that altered immune system function may be a common causal link. If so, the immune model proposes a plausible framework by which to investigate co-morbidity between synesthesia and other conditions. If this hypothesis is correct, we might ask whether the prevalence of synaesthesia is also higher in populations with other autoimmune conditions. Indeed, recent data from our lab has led us to explore whether developmental synaesthesia might occur more prevalently in people with the radiological profile of multiple sclerosis (MS), for example, a demyelinating disease of the human CNS (Simner et al., submitted). A maladaptive immune system is an undisputed factor in the pathogenesis of MS (Trapp and Nave, 2008), and the majority of genes implicated in MS have an immune function (Gourraud et al., 2012). The immune hypothesis of synaesthesia might therefore lead us to investigate whether synaesthesia and autoimmune conditions such as MS could share overlapping genetic origins in contributing to cortical development and immune function.

CONCLUSION AND FUTURE DIRECTIONS

We have proposed that CNS/immune system interactions during early life may play a role in the development of synesthesia. We have asked whether genes with dual functionality in brain development and immunity may be at the origin of existing models of synesthesia, and this mechanism would provide a framework to investigate associations between synesthesia and other immune-related conditions. We make our proposal here as a model for developmental synesthesia, although not all cases of synesthesia are developmental in nature. Synesthesia may also be acquired, for example as a result of brain injury (Schweizer et al., 2013) or induced by consumption of psychoactive drugs such as Lysergic acid diethylamide (LSD; e.g., Cytowic, 1989). Our hypothesis does not speak directly to such cases, and it is not yet known whether these different

forms of synesthesia have the same neural origins or mechanisms. It is interesting to note however that immune system activity is elevated after brain injury, and processes such as apoptosis do become activated (Griffiths et al., 2010). It is therefore at least plausible to ask whether the immune system might also play a role in the appearance of non-developmental synesthesias. Identification of genes that contribute to the development of synesthesia will make a significant contribution to the validity of this hypothesis, and whether synesthesia has one cause or many.

ACKNOWLEDGMENTS

We are grateful to Richard Shillcock for useful discussions, Lisa Boulanger for insightful suggestions and feedback and the anonymous reviewers for helpful comments on the manuscript. Duncan A. Carmichael was supported in part by grants EP/F500385/1 and BB/F529254/1 for the University of Edinburgh, School of Informatics Doctoral Training Centre in Neuroinformatics and Computational Neuroscience (www.anc.ac.uk/dtc) from the UK Engineering and Physical Sciences Research Council (EPSRC), UK Biotechnology and Biological Sciences Research Council (BBSRC), and the UK Medical Research Council (MRC).

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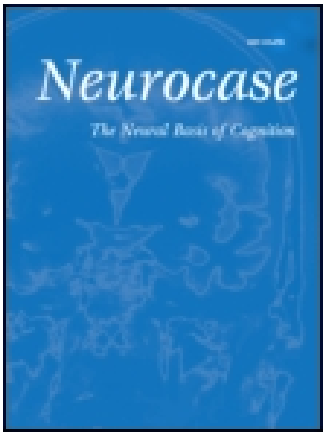
Received: 31 July 2013; accepted: 23 August 2013; published online: 11 September 2013.

Citation: Carmichael DA and Simner J (2013) The immune hypothesis of synesthesia. Front. Hum. Neurosci. 7:563. doi: 10.3389/fnhum.2013.00563

This article was submitted to the journal Frontiers in Human Neuroscience.

Appendix F

Rates of white matter hyperintensities compatible with the radiological profile of multiple sclerosis within self-referred synesthete populations



Neurocase: The Neural Basis of Cognition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/nncs20

Rates of white matter hyperintensities compatible with the radiological profile of multiple sclerosis within self-referred synesthete populations

Julia Simner[a], Duncan A. Carmichael[abc], Edward M. Hubbard[d], Zoe Morris[e] & Stephen M. Lawrie[c]

[a] Department of Psychology, University of Edinburgh, Edinburgh, UK
[b] Institute for Adaptive and Neural Computation, University of Edinburgh, Edinburgh, UK
[c] Division of Psychiatry, University of Edinburgh, Royal Edinburgh Hospital, Edinburgh, UK
[d] Department of Educational Psychology, University of Wisconsin-Madison, Madison, UK
[e] Division of Clinical Neurosciences, Western General Hospital, Edinburgh, UK

Published online: 05 Mar 2014.

To cite this article: Julia Simner, Duncan A. Carmichael, Edward M. Hubbard, Zoe Morris & Stephen M. Lawrie (2015) Rates of white matter hyperintensities compatible with the radiological profile of multiple sclerosis within self-referred synesthete populations, Neurocase: The Neural Basis of Cognition, 21:3, 322-330, DOI: 10.1080/13554794.2014.892625

To link to this article: http://dx.doi.org/10.1080/13554794.2014.892625

Neurocase, 2015
Vol. 21, No. 3, 322–330, http://dx.doi.org/10.1080/13554794.2014.892625

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Rates of white matter hyperintensities compatible with the radiological profile of multiple sclerosis within self-referred synesthete populations

Julia Simner[a]*, Duncan A. Carmichael[a,b,c], Edward M. Hubbard[d], Zoe Morris[e] and Stephen M. Lawrie[c]

[a]*Department of Psychology, University of Edinburgh, Edinburgh, UK;* [b]*Institute for Adaptive and Neural Computation, University of Edinburgh, Edinburgh, UK;* [c]*Division of Psychiatry, University of Edinburgh, Royal Edinburgh Hospital, Edinburgh, UK;* [d]*Department of Educational Psychology, University of Wisconsin-Madison, Madison, UK;* [e]*Division of Clinical Neurosciences, Western General Hospital, Edinburgh, UK*

(*Received 1 October 2013; accepted 28 January 2014*)

Synesthesia is an inherited condition causing unusual secondary sensations (e.g, sounds might be experienced as both auditory and visual percepts). The condition has been linked with cognitive and perceptual benefits and is considered a benign alternative form of perception. Here, we investigate self-referred synesthete populations and their rates of radiologically determined white matter hyperintensities (WMH) of a type compatible with the McDonald imaging criteria for the diagnosis of multiple sclerosis (MS). MS is a chronic condition resulting in damage to myelination surrounding nerve fibers of the central nervous system (CNS). Magnetic resonance imaging (MRI) features highly suggestive of MS without overt clinical symptoms are termed *radiologically isolated syndrome* (RIS). We present data showing that the shared MRI profile of MS and RIS has been significantly overrepresented in synesthetes who have participated in neuroimaging research. We present validation of the clinical and MRI status of these synesthetes and an analysis showing the significant probability their unusual numbers may not have arisen by chance. We discuss how to interpret significant data based on small case numbers and consider the implications of our findings for synesthesia's clinical status.

Keywords: synaesthesia; synesthesia; multiple sclerosis; radiologically isolated syndrome; comorbidity

For people with synesthesia, stimuli are experienced with unusual secondary associations. For example, sound-color synesthetes experience sound stimuli as accompanied by both auditory and visual (i.e., color) percepts (Asher et al., 2009). Synesthesia tends to be regarded in positive rather than negative terms and has a range of cognitive and perceptual benefits (e.g., for memory; Yaro & Ward, 2007). Here, we investigate an observation of unusually high rates of white matter hyperintensities (WMH) in a group of synesthetes. These hyperintensities are areas of high intensity on T2-weighted magnetic resonance imaging (MRI) scans of a type compatible with the McDonald (imaging) criteria for the diagnosis of multiple sclerosis (MS; Polman et al., 2011).

MS is an inflammatory disease of the central nervous system (CNS) with a broad range of perceptual and motor symptoms. It is thought to be caused by environmental risk factors in combination with genetic susceptibility (Compston & Coles, 2008). MS is characterized by demyelination, axonal loss, and gliosis of white matter (Weiner, 2009). Diffusion tensor imaging (DTI) shows reduced fractional anisotropy (FA) scores in MS patients, indicating reduced white matter integrity in lesion sites and elsewhere (Roosendaal et al., 2009). Although MS is typically classified as a white matter disorder, gray matter is also affected, with reduced volume common in several areas (Ceccarelli et al., 2008). If patients present with MRI features suggestive of MS but without overt clinical symptoms, this is termed *radiologically isolated syndrome* (RIS). From a radiological perspective, MRI scans of RIS and MS are indistinguishable, and it is only the presence of clinical symptoms that differentiates between the two conditions. There is also a clinical relationship between the conditions in that approximately one-third of patients with RIS develop MS symptoms within 2–5 years (Granberg, Martola, Kristoffersen-Wiberg, Aspelin, & Fredrikson, 2013).

Structural differences in white and gray matter also characterize the condition of synesthesia. Synesthetes show both increases and decreases in gray matter volume (Hänggi, Beeli, Oechslin, & Jäncke, 2008; Jäncke, Beeli, Eulig, & Hänggi, 2009) and altered coherence of white matter (Hänggi, Wotruba, & Jancke, 2011; Jäncke et al., 2009; Rouw & Scholte, 2007). This altered white matter is found in regions implicated by the synesthetic report and also elsewhere. For example, synesthetes experiencing

*Corresponding author. Email: j.simner@ed.ac.uk
JS and DAC contributed equally to this work. JS and EMH conceived the project; JS and DAC wrote the paper with team feedback; JS, DAC, EMH and SML gathered data, ZM assessed and interpreted neuroradiological data, and JS and DAC conducted the statistical analyses.

color sensations show increased FA compared with controls near color selective region V4 (Rouw & Scholte, 2007) but also show FA increases (Rouw & Scholte, 2007) and decreases (Hänggi et al., 2008) in parietal lobe. The cortical reorganization of white and gray matter in synesthesia might call for comparison with MS-RIS, and we discuss in this article whether epidemiological or pathological links might exist between them.

Our question is motivated by a particularly high occurrence of MRI abnormalities consistent with the neuroradiological profile of MS-RIS within synesthetes who have presented to our laboratories for brain imaging studies. In three independent synesthete cohorts across three countries (San Diego, CA, United States; Paris, France; and Edinburgh, United Kingdom), we have opportunistically found clinical and radiological indicators suggestive of MS in 1 in 6 synesthetes, 1 in 10 synesthetes, and 1 in 13 synesthetes, respectively. Our studies were not initiated with the intention of investigating the prevalence of MS-RIS, but our unexpected findings have led us to evaluate the post hoc detection of white matter abnormalities in these three participants. All three affected cases met the McDonald (imaging) criteria for the diagnosis of MS. Subject 1 (United States) has a full diagnosis of MS, while Subjects 2 (France) and 3 (United Kingdom) are currently free from clinical symptoms and therefore considered to have RIS. Subjects 2 and 3 were initially flagged by routine protocols in our studies in which neuroradiologists examine T2-weighted axial MRI scans for unanticipated pathology. Subject 1's diagnosis was first suggested by her general practitioner several months after our study, because routine radiological checks were not part of that study's protocol.

Our finding of three cases of MS-RIS among 29 synesthetes is suggestive of an unusually high rate. However, we must address the possibility that we have inadvertently focused on just those studies where anomalies were found, rather than all synesthesia imaging studies to date. Collectively, 29 published studies (see Table 2) have scanned 211 synesthetes (including 6 of our own 29 synesthetes, described in Hubbard, Arman, Ramachandran, & Boynton, 2005). Together with our remaining unpublished cases (N = 23), this gives 234 synesthete scans in existence known to us. Outside our cohort of 29 participants, we have been able to ascertain that 80 additional synesthetes' MRI scans were checked with a similar radiologist protocol, and none revealed anomalous findings of this type.[1] A further 121 were scanned without this protocol and the remaining four scans have an unknown status. The most conservative approach is to assume no cases of pathology in any of the 234 scanned cases, other than the three identified here. Our methodology discussed later is to evaluate this occurrence of MS-RIS in synesthetes against appropriate baselines. We first begin with full case descriptions of the three affected synesthetes. This is followed by a set of analyses that consider the appropriate baselines against which to compare our finding of three affected cases (1 MS; 2 RIS). To anticipate our methodologies discussed later, we take the most stringent standards against which to compare our observations (given factors such as the geographic variability of MS and the sampling method of our studies) and nonetheless find that the number of affected cases is significantly higher than chance would predict.

Empirical study

In this section, we evaluate our three cases of MS-RIS both qualitatively and quantitatively, beginning with a detailed clinical evaluation.

Case details of affected synesthetes

Synesthesia status

Table 1 shows the synesthetic status and demographic background of the three synesthete participants in whom radiological anomalies have been identified. The table shows their synesthesia phenotypes, and these comprise the following: *grapheme-color synesthesia* gives rise to colored percepts triggered by letters or digits (e.g., the letter *A* might be red; Asher et al., 2009); *sequence-*

Table 1. Synesthetic Case Descriptions.

Test site	Age	Sex	Nationality	Synesthesia phenotype(s)*	MS-RIS?
San Diego	26	Female	American	Grapheme-color	MS
Paris	25	Female	French	Number-space, time-space (colored), grapheme-color	RIS
Edinburgh	31	Female	British/English	Sequence-personality, letter-space, numbers-space, time-space	RIS

*Verification of synesthesia relies on the behavioral "gold standard" test for synesthesia, which assesses the consistency of the synesthetic report over time (e.g., Eagleman, Kagan, Nelson, Sagaram, & Sarma, 2007; Simner et al., 2006). In this, participants are required to first report their synesthetic experiences for a list of provided stimuli (e.g., they report their colors for a list of letters), and they are subsequently retested after some considerable time (e.g., 6 months, for the case scanned in San Diego; see Hubbard et al., 2005 for methodological details). Synesthetes are identified as those who are significantly more consistent in their reports over time, compared to a group of matched controls who invent/recall analogous associations.
Note: For each affected synesthete, the table shows the imaging lab testing site, the participant's age at scanning, sex, country of origin, verified synesthesia phenotype(s), and the clinical status.

personality synesthesia gives rise to complex personifications triggered by the members of linguistic sequences (e.g., *Monday* might be "female, unfriendly"; Simner & Holenstein, 2007); *number-space synesthesia*, *letter-space synesthesia* and *time-space synesthesia* are all variants of the broader category of *sequence-space synesthesia*, in which linguistic sequences are perceived in spatial arrays (e.g., the letters A–Z might extend in an undulating line from right to left across the visual field; see Simner, 2009 for review).

Clinical status of MS-RIS

All three synesthete participants in question met the McDonald (imaging) criteria for the diagnosis of MS. This requires the presence of one or more T2-weighted high signal lesions in at least two of the following four areas of the CNS: periventricular, juxtacortical, infratentorial, or spinal cord. A full diagnosis of MS has been given in the case of one participant, while the other two subjects are currently free from clinical symptoms and are therefore considered to have RIS. The synesthetic status of each participant is shown in Table 1 and their clinical status is described here.

Subject 1 (San Diego, CA, United States) was diagnosed with clinically definite relapsing-remitting MS several months after taking part in a synesthesia study in 2001. A diagnosis of MS was first suggested by this participant's general practitioner because routine radiological checks were not part of the study's protocol. Subsequent neurological follow-up consisting of sagittal and axial T1-weighted FLAIR and axial T2-weighted and axial and coronal T1-weighted MRI scans revealed extensive hyperintensities in both the brain and cervical spine, considered to be pathognomonic for MS. These white matter lesions, in conjunction with history of clinical symptoms, confirmed this participant's diagnosis of MS. This participant had a total of 16 lesions, in both periventricular and juxtacortical white matter (see Figure 1). Periventricular lesions were located in the corpus callosum and in occipital and parietal areas. Juxtacortical lesions were found in the parietal region. Lesions were also detected in the spinal cord. This subject was originally scanned for, but subsequently excluded from, Hubbard et al. (2005) (see Table 2).

Subject 2 (Paris, France) participated in synesthesia research in 2006, where her axial T2-weighted MRI scan was reviewed as part of routine assessments for unanticipated pathology by a consultant neuroradiologist. Initial examination of her resultant T2-weighted MRI scan revealed WMH in the brain. A second, axial T2-weighted FLAIR MRI scan, obtained 2 years after the initial scan, confirmed the presence of white matter lesions, judged to be consistent with McDonald criteria (see Figure 2). Due to a lack of progression, these lesions are considered stable and this participant has remained free of clinical symptoms. This participant had more than 20 identified lesions in periventricular and juxtacortical white matter. Periventricular lesions were in frontal and parietal areas, while juxtacortical lesions were distributed across frontal, parietal, and temporal regions. This subject was scanned for Hubbard et al. (unpublished data) (see Table 2).

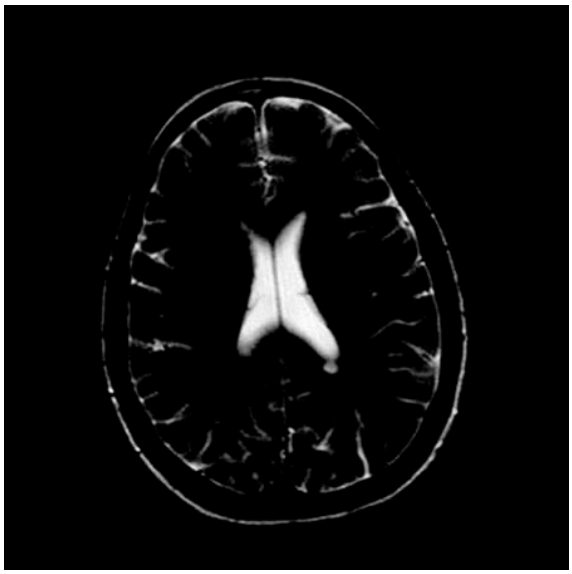

Figure 1. Axial T2-weighted image showing a left parietal ovoid periventricular lesion (of 16 overall lesions), from subject 1.

Subject 3 (Edinburgh, United Kingdom) participated in synesthesia research in 2008 and her resultant axial T2-weighted MRI scan was also examined routinely by a consultant radiologist to detect unanticipated pathology. Initial examination revealed WMH in the brain of this subject. This T2-weighted MRI scan was independently examined by a second neuroradiologist who confirmed that the number and location of the white matter lesions met the McDonald imaging criteria for diagnosis of MS (see Figure 3). Again, this participant has remained free of clinical symptoms since the initial presentation of her abnormal scan. This participant had more than 20 lesions in periventricular and juxtacortical white matter (see Figure 3). Periventricular lesions were found in frontal, parietal, temporal, and occipital areas. Juxtacortical lesions were identified in temporal and parietal areas. Infratentorial lesions were also present. This subject was scanned for, and subsequently excluded from, a study by Rehme et al. (in review) (see Table 2).

Case details of all synesthetes with existing MRI scans

Table 2 shows what is to our knowledge all studies that have generated MRI scans from synesthete participants at the time of writing including all published studies, plus our two unpublished samples. Published articles were

Table 2. MRI studies of synesthete participants.

Year	Authors	Total number of participants (*N*)	Status‡‡	Number of female participants (*N*)
European studies				
2001	Aleman et al.	1	¥	1
2001	Weiss et al.	1	†	1
2005	Weiss et al.	9	†	6
2006	Hubbard et al. (unpublished data)	10	§	10
2006	Sperling et al.	4		4
2007	Rouw and Scholte	0*		0
2008	Hänggi et al.	1	¥	1
2009	Jäncke et al.	0*		0
2009	Weiss and Fink	16*	†	15
2010	Rouw and Scholte	42	¥	42
2010	van Leeuwen et al.	21	¥	19
2011	Gaschler-Markefski et al.	7	¥	6
2011	Hänggi et al.	24	¥	20
2011	Specht and Laeng	2	††	2
2011	van Leeuwen et al.	0*		0
2012	Dovern et al.	5*	†	5
2012	Hupe et al.	10	¥	7
2012	Neufeld et al.	14	¥	9
UK studies				
2002	Nunn et al.	13	††	13
2005	Blakemore et al.	1	††	1
2006	Gray et al.	2*	††	2
2007	Cohen Kadosh et al.	1	††	0
2008	Bor et al.	1	††	0
2008	Tang et al.	10	††	8
2011	Jones et al.	2	†† ¶	1
2012	Banissy et al	9	†	5
2013	Rehme et al. (under review)	13	§	13
N. American studies				
2003	Elias et al.	1	¥	1
2005	Hubbard et al.	6	§	3
2012	Brogaard et al.	1	††	0
Other studies				
2006	Rich et al.	7	††	6
	Total	234		201

*Subject numbers have been adjusted to exclude duplicate participants already scanned in previous studies by the same group (shown elsewhere in the table).
†Routine radiological checks for pathology performed by neurologist and no anomalies found.
††Routine radiological checks carried out by a neuroradiologist and no anomalies found.
§Members of our study cohort (i.e., studies directed in our labs); three anomalies in $N = 29$ scans.
¶A study directed by colleagues outside our labs, but JS coauthoring.
¥No routine radiological checks for pathology performed.
‡‡Status with respect to radiological anomalies (see the previous footnotes; unknown unless otherwise stated).

retrieved from an all-years search of PubMed using search terms "syn*esthesia" (UK/US spellings) and "MRI", and any additional details on participants given in the following discussion that were not available in the literature were retrieved by contact with the authors of these studies.

Analysis of the prevalence of MS-RIS cases among scanned synesthetes against expected baselines

We found three cases of MS-RIS in 234 synesthetes who were self-referred for brain scanning studies across the literature. To establish whether the number of affected synesthetes is statistically significant, we must compare the prevalence of observed cases against a meaningful baseline. Since RIS and MS are indistinguishable, from a radiological perspective, we first consider all three cases as a unified phenomenon. Then, since RIS and MS are different in a clinical/symptomatic sense, we additionally consider the two cases of RIS as a distinct phenomenon. Methodologically speaking, we will take our RIS baseline from a meta-analysis of the prevalence of RIS across all MRI scans described in the imaging literature (and later we describe our very conservative approach in this regard). We will take our MS baselines from rates reported

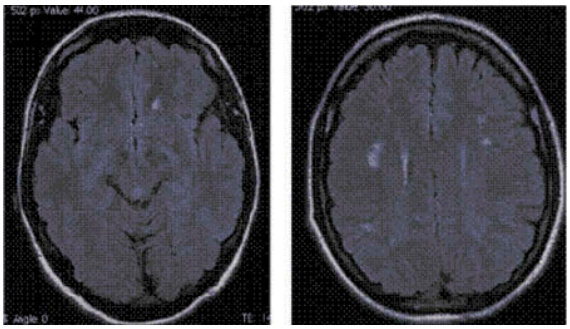

Figure 2. Axial T2-weighted FLAIR images showing (left) a left frontal periventricular lesion and (right) multiple lesions; the right parietal lesion involves juxtacortical U fibers. [To view this figure in color, please see the online version of this Journal.]

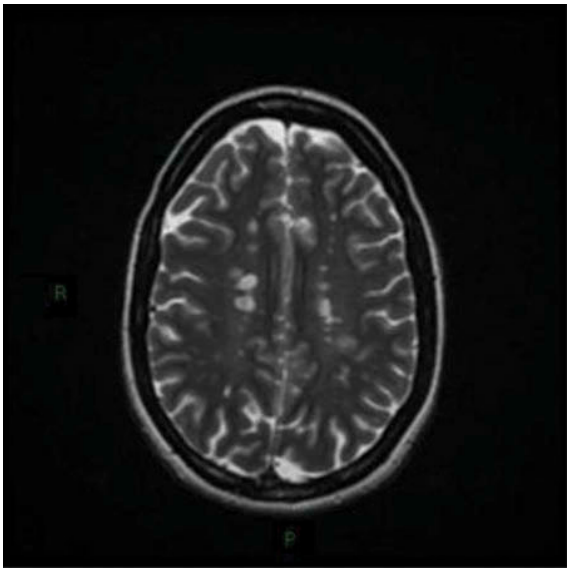

Figure 3. Axial T2-weighted image showing multiple ovoid periventricular white matter lesions, from subject 3.

in clinical prevalence studies. Since the prevalence of MS is sex-linked and geographically variable, we have considered that the large majority of all scanned synesthetes are female (86%) and have come from studies conducted in Europe (94%). Accordingly, we took a female MS prevalence figure for Europe. Again however, since a key aim was to be maximally conservative (i.e., to compare our findings with the highest prevalence rates where possible), we additionally reanalyzed our data using the most stringent prevalence rate according to the nationality of the three affected cases (American/French/English). In other words, since the rate of MS is highest in England (vs. America or France), we used this highest England baseline in a second analysis. In the following we describe these baseline selection procedures in more detail and the resultant statistical outcomes of our analyses (showing whether MS-RIS is indeed significantly higher in self-referred synesthete samples).

Baseline selection and results

There is no published combined prevalence of MS-RIS, so we use an additive value from each separately. Our recent meta-analysis (Morris et al., 2009) shows a baseline of RIS in the general population of 57.8/100,000. This figure was based on nine cases of unanticipated WMH considered as definite demyelination, which were found in 15,559 scans reported in the literature. To be conservative here, we will also include three cases of *possible* demyelination in that meta-analysis, and furthermore, we will consider scans only from self-referred volunteer research participants (giving a total of 12 RIS cases found in 8,441 research scans; Proportion (P) = 0.14%, 95% CI [0.08%, 0.25%]; Morris et al., 2009). We consider only self-referred volunteers because this type of participant is similar to those in our own cohort of synesthetes and across the 234 scanned synesthetes more widely. It is important to consider this method of self-referral since it might increase the number of RIS cases in our cohort. Specifically, self-referred/volunteer recruitment in any brain imaging study may increase rates of neuropathology by over-recruitment of participants who are seeking covert evaluation for undeclared neurological complaints (Morris et al., 2009). In other words, our three affected synesthetes may have volunteered for our studies to assuage personal neurological concerns, and we must therefore compare their number against a baseline that specifically takes this into account. In summary, all these considerations give us a comparative baseline for RIS in the general population of 142/100,000. However, we point out that this baseline is likely to be highly inflated since closer inspection of the data that contributed to our meta-analysis reveals that two-thirds of cases contributing to this figure were found in a single study in which 90.0% of participants were former lead workers with a mean age of 60.1 years (Alphs, Schwartz, Stewart, & Yousem, 2006). Since the likelihood of detecting WMH increases significantly with age (Smith, Snowdon, Wang, & Markesbery, 2000) and with exposure to neurotoxicants such as lead (Stewart et al., 2006), the true prevalence of WMH in the general population of self-referred volunteers is likely to be substantially lower than the baseline we are selecting here. Nonetheless, we use this baseline as a maximally conservative estimate (to combine with a suitable estimate for MS prevalence below), for comparison with our synesthete sample.

The prevalence of MS in the general population is better understood and known to vary by geographic region (with particularly high rates in Scotland, Sutherland, 1956, and by sex: females approximately 2:1; Pugliatti et al., 2006). This is of note given our Edinburgh testing study

(in which 4 of 13 participants were Scottish; the remainder traveled from England) and the fact that the scanned synesthesia population is skewed towards females (F = 201) – as potentially, is synesthesia in general (e.g., see Ward & Simner, 2005). Considering the sex and nationality of the 234 scanned synesthetes (using the location of testing centers as an indicator of nationality for cases unknown to us), an appropriate baseline is the rate of MS in female Europeans (110/100,000[2]) since synesthetes were virtually exclusively European (94%, N = 220; including four Scots, none of whom showed pathology). This we combined with the baseline for RIS to give an additive RIS-MS baseline of 252/100,000.

Using the exact binomial test, we calculated the probability of obtaining our observed number of MS-RIS cases within scanned synesthetes, given the expected baseline prevalence estimates generated earlier. Thus, we estimated the probability of three observations of MS-RIS among 234 self-referred synesthetes (Proportion (P) = 1.3%, 95% binomial CI [0.3%, 3.7%]; 1282/100,000) given an expected population prevalence of 252/100,000. Our statistical test shows this rate among synesthetes to be significantly higher than chance might predict (p = 0.02). This difference remains significant (p = 0.04) even if we substitute the female Europe MS rate and use instead the female England MS rate,[3] as the most extremely conservative option given the demographics of our three affected cases (American/French/English).

Combining our three cases has allowed us to consider the shared MRI profile of MS and RIS and their related clinical progression (Granberg et al., 2013). However, we might alternatively exclude our case of MS as a separate phenomenon and consider only our two observations of RIS against all 103 scans with protocols to check for such anomaly (P = 1.9%, 95% binomial CI [0.2%, 6.7%]). Taking again our highest RIS baseline (142/100,000), this finding is also highly significant (p = 0.01). Indeed, taking even the most extreme assumption of no other cases in any synesthete scanned to date – checked or unchecked – an observation of two cases in 234 (P = 0.9%; binomial CI [0.1%, 3.1%]) remains significant (p = 0.04).[4]

Discussion

We initially observed three cases showing WMH compatible with the radiological profile of MS – one case of MS and two of RIS – in 29 synesthetes from three of our imaging labs across three different countries. We have taken the most conservative approach in assuming no further cases in any synesthetes scanned to date, placing the prevalence at 3 affected cases in 234 synesthetes (1,282/100,000, compared to a population baseline estimate of 252/100,000). Two cases of RIS in 103 brain scans checked for pathologies would, if representative, place the prevalence of RIS in synesthetes at 1,942/100,000 (compared to a baseline of 142/100,000). These rates are significantly higher than expectation, even against our highly conservative baselines. We also specifically controlled for the possibility that our three affected cases may have volunteered for our studies to assuage personal neurological concerns. We did this by comparing our RIS cases to a baseline constructed only from studies that included similar, self-referred, volunteer participants. In other words, we compared our rates to studies likely to have just as many "concerned self-referrers" as our own, rather than to studies using nonvoluntary recruitment methods (e.g., work-related health screening; Weber & Knopf, 2004).

We would like to very clearly acknowledge that we report only a small number of affected cases, and we evidently do not claim that synesthesia causes MS-RIS. Indeed, our small sample size means we hold back from making any strong claim whatsoever about links between these two conditions. There are many environmental factors thought to contribute to the development of MS and RIS; the evidence we present here suggests having synesthesia may be one factor that could merit further investigation. Indeed, we have chosen to present our data for two reasons. The first is that the rates we have found are statistically significant; a considerably greater sample size would usually be needed to detect the numbers we have found – although again our sample sizes are small. The second reason we present our data is a practical one: cases of pathology are usually excluded from MRI study populations as soon as they are detected. Therefore, they often do not appear in the literature and so remain unknown outside the research group. We have published our data because we judge it important that other researchers working in this area are aware of our cases, and so might not overlook future neurological abnormalities, should they ever be discovered. If a meaningful link between synesthesia and MS did exist, and as scanning of synesthetes for research purposes becomes more commonplace, further cases of this nature would arise, and so we encourage researchers to make any cases known to the wider community if they share similarities with our own.

One explanation for a link between synesthesia and MS could be the occurrence of synesthesia-like symptoms *after* the onset of MS. In other words, it is possible that the degenerative neurological damage caused by MS might give rise to sensory disorders that *mimic* synesthesia, while having different causes. Sensory disturbances do accompany MS (e.g., changes in color vision; Gregori, Papazachariadis, Farruggia, & Accornero, 2011) although this hypothesis would imply that the onset of synesthesia-like symptoms should be later in life – resulting from MS-related changes in brain structure. However, we do not believe this accounts for the cases we present here and for two reasons. Later-acquired synesthesias are qualitatively different to developmental variants (Ward, 2013) and do

not reflect the synesthesias of our participants. Acquired variants of synesthesia tend to involve low-level sensory triggers (e.g., tones) rather than learned symbols such as graphemes (Ward, 2013) although it is precisely this latter type of trigger (graphemes etc.) that our own cases possess – and which might therefore be considered a hallmark of developmental synesthesia. Furthermore, all three of our cases report life-long synesthesia, stemming back from early childhood, and being present for as long as they can remember.

Finally, we point out one recent finding that might be considered alongside our own. Bashir, Lipton, Ashina, and Ashina (2013) have shown white matter abnormalities in people suffering migraine, especially those for whom the migraine is accompanied by auras. Migraine has been linked with synesthesia (e.g., Alstadhaug & Benjaminsen, 2010) and the auras associated with migraine are visual disturbances that themselves might be considered as resembling certain types of synesthetic sensations (e.g., colored photisms). Future studies might therefore further explore any possible links between synesthesia and migraine and the types of visual disturbances they each engender.

In conclusion to the current study, we have demonstrated an apparent statistical link between MS-RIS and synesthesia. Overall, we have been conservative in our study in three ways: we selected baselines in an overly conservative way, we took additional measures to be conservative when conducting our statistical analysis (e.g., assuming no anomalies in scans *not* assessed by radiologists), and we are circumspect in the interpretation of our findings. Because our study relies on a small number of cases – three only – we do not claim that any causal link exists between synesthesia and MS-RIS, and we present our findings with this strong caveat. Nonetheless, we present these significant data so that imaging researchers might consider them when evaluating any anomalies that may arise in future studies. If our epidemiological findings are indeed later supported by additional evidence, this could invite a debate about the clinical status of synesthesia, which has previously been associated with largely beneficial rather than unfavorable characteristics (but see Carruthers, Miller, Tarrier, & Whorwell, 2012). To investigate this hypothesis further, we are currently also exploring whether developmental synesthesia is found in high numbers within populations of people with MS, which would enable firmer conclusions to be drawn regarding the validity or otherwise of the statistical associations we report here.

Acknowledgments

We are grateful to Daisy Mollison for her radiological expertise, to Richard Shillcock for his feedback on the manuscript, to Linda Williams (Centre for Population and Health Sciences) for her epidemiological and statistical guidance, and to Catherine Oppenheim and Lucie Hertz-Pannier for their help retrieving archived data. The authors declare no competing interests.

Funding

DAC was supported in part by grants [grant number EP/F500385/1], [grant number BB/F529254/1] for the University of Edinburgh, School of Informatics Doctoral Training Centre in Neuroinformatics and Computational Neuroscience (www.anc.ac.uk/dtc) from the UK Engineering and Physical Sciences Research Council (EPSRC), UK Biotechnology and Biological Sciences Research Council (BBSRC), and the UK Medical Research Council (MRC).

Notes

1. Where MS-RIS anomalies were found, authorship was invited for the current article and this would explain why three cases were found in our own cohort but not elsewhere.
2. Calculation of the rate of female MS in Europeans: Pugliatti et al. (2006) report that "the total estimated prevalence rate of MS [in Europe] for the past three decades is 83 per 100 000 with … a female: male ratio around 2.0" (p. 700). On this basis, and given the overall population sex ratio in the European Union (0.96 males to each female; The World Factbook, 2011, Washington, DC: Central Intelligence Agency, 2011), we calculate the estimated European female MS rate at 110/100,000.
3. Calculation of the rate of female MS in England: There was no available data for female MS prevalence in England per se and so we calculated this based on the rate of MS in eastern England (153/100,000; the highest regional rate in England reported by Compston et al., 2006) and the male: female ratio of MS (2:1; Compston et al., 2006) in combination with the overall population prevalence of women versus men in the United Kingdom (1.01:1; The World Factbook, 2011, Washington, DC: Central Intelligence Agency, 2011). This gives an estimate of female MS in England at 204/100,000.
4. Analyzing the RIS cases and MS case separately serves an additional purpose. The MS case was the first reported case and as such can be viewed as the observation that led to the forming of our hypothesis. It could be argued that including this case in subsequent analyses, especially given the small numbers of cases involved, may lead to inappropriate conclusions being drawn. It is important to point out therefore that when this initial case is excluded and the subsequent two cases are analyzed, the outcome remains statistically significant, adding further methodological rigor for this hypothesis.

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Appendix G

Validating a standardised test battery for synesthesia: Does the Synesthesia Battery reliably detect synesthesia?

Consciousness and Cognition 33 (2015) 375–385

Contents lists available at ScienceDirect

Consciousness and Cognition

journal homepage: www.elsevier.com/locate/concog

Validating a standardised test battery for synesthesia: Does the Synesthesia Battery reliably detect synesthesia?

D.A. Carmichael [a,b,c,e,*], M.P. Down [b], R.C. Shillcock [a,b], D.M. Eagleman [d], J. Simner [a,e]

[a] Dept. of Psychology, University of Edinburgh, 7 George Square, EH8 9JZ, UK
[b] Institute for Adaptive & Neural Computation, University of Edinburgh, 10 Crichton Street, EH8 9AB, UK
[c] Division of Psychiatry, University of Edinburgh, Royal Edinburgh Hospital, EH10 5HF, UK
[d] Department of Neuroscience, Baylor College of Medicine, Houston, TX, USA
[e] School of Psychology, University of Sussex, Falmer BN1 9QH, UK

ARTICLE INFO

Article history:
Received 6 March 2014
Available online 28 February 2015

Keywords:
Synesthesia
Validation
Test
Assessment
Consistency
Prevalence
Grapheme-colour

ABSTRACT

Synesthesia is a neurological condition that gives rise to unusual secondary sensations (e.g., reading letters might trigger the experience of colour). Testing the consistency of these sensations over long time intervals is the behavioural gold standard assessment for detecting synesthesia (e.g., Simner, Mulvenna et al., 2006). In 2007 however, Eagleman and colleagues presented an online 'Synesthesia Battery' of tests aimed at identifying synesthesia by assessing consistency but within a single test session. This battery has been widely used but has never been previously validated against conventional long-term retesting, and with a randomly recruited sample from the general population. We recruited 2847 participants to complete The Synesthesia Battery and found the prevalence of grapheme-colour synesthesia in the general population to be 1.2%. This prevalence was in line with previous conventional prevalence estimates based on conventional long-term testing (e.g., Simner, Mulvenna et al., 2006). This reproduction of similar prevalence rates suggests that the Synesthesia Battery is indeed a valid methodology for assessing synesthesia.

1. Introduction

Synesthesia is an inherited condition in which everyday stimuli trigger unusual secondary sensations. For example, synesthetes listening to music might see colours in addition to hearing sound (Ward, Huckstep, & Tsakanikos, 2006). One particularly well-studied variant is *grapheme-colour synesthesia*, in which synesthetes experience colours when reading, hearing or thinking about letters and/or digits (e.g., Simner, Glover, & Mowat, 2006). Despite being first reported over two hundred years ago (by Sachs, 1812; see Jewanski, Day, & Ward, 2009) synesthesia was initially an under-researched and poorly-understood area of human experience until the last decades of the 20th century. A significant factor in the elevation of synesthesia as a tractable topic was the realisation – and subsequent empirical confirmation – that synesthetes' experiences could be verified behaviourally by the fact that they remain conspicuously stable over time (Baron-Cohen, Wyke, & Binnie, 1987; Jordan, 1917). Specifically, synesthetes tend to be highly consistent when reporting their synesthetic sensations for any given stimulus. For example, if the letter J triggers the colour pale blue for a given synesthete, she will tend to repeat that J is pale blue (not green, not yellow, etc.) when repeatedly tested over days, months and even years. Indeed,

* Corresponding author at: 3.34 Informatics Forum, University of Edinburgh, 10 Crichton St, Edinburgh EH8 9AB, UK.
E-mail address: d.a.carmichael@ed.ac.uk (D.A. Carmichael).

http://dx.doi.org/10.1016/j.concog.2015.02.001
1053-8100/

one study was able to show that synesthetic sensations had remained consistent over at least three decades (Simner & Logie, 2008).

This stability of responses over time is considered one of the central features of synesthesia and is routinely verified in almost every publication on the subject (e.g., Asher, Aitken, Farooqi, Kurmani, & Baron-Cohen, 2006; Baron-Cohen, Burt, Smith-Laittan, Harrison, & Bolton, 1996; Rich, Bradshaw, & Mattingley, 2005; Ward & Simner, 2003; but see Simner, 2012). In other words, while a wide range of behavioural approaches have been employed to assess the nature of the synesthetic experience, experimental methodologies aiming to *validate* synesthesia have almost exclusively focussed on the feature of consistency. Hence, researchers selecting synesthete participants for study first verify the genuineness of each case by requiring their synesthetes to demonstrate high levels of consistency over time compared to non-synesthete controls (e.g., Asher et al., 2006; Baron-Cohen et al., 1996; Simner et al., 2006). Controls are tested on analogous associations (i.e., they invent colours for the 26 letters, say, and then attempt to recall these colour associations later) and typically perform significantly worse than synesthetes.

Although more than a hundred contemporary studies rely on this test of consistency for genuineness, the particular instantiation of the test has varied widely. For example, a wide range of methods have been used to elicit synesthetic colours: participants have indicated these by either giving verbal descriptions (e.g., Ward, Simner, & Auyeung, 2005), written descriptions (Simner, Glover et al., 2006), using Pantone© swatch colour charts (Asher et al., 2006), electronic colour charts (Simner, Harrold, Creed, Monro, & Foulkes, 2009) or even computerised colour pickers offering extensive palettes of >16 million colours (e.g., Simner & Ludwig, 2012). In this way, synesthesia research has used varying methods, which in turn might raise difficulties for researchers when trying to meaningfully compare data.

Despite this superficial variability however, the test of genuineness has nonetheless tended to rely on one key shared feature: synesthetes must outperform controls over fairly lengthy re-test intervals. Consider, for example, the most widely cited large-scale screening for synesthesia (Simner, Mulvenna et al., 2006) in which a large sample of participants were opportunistically recruited from the communities of Edinburgh and Glasgow Universities, and individually assessed for synesthesia. Participants first indicated by questionnaire whether they believed they experienced synesthesia, and those who reported in the affirmative were asked to provide their synesthetic associations (e.g., the colours of letters). These participants were then retraced after considerable time had passed (on average 6.0 months) and were asked in a surprise retest to re-state their associations. A group of controls without synesthesia performed an analogous task but were re-tested after only two weeks. Synesthetes were able to significantly out-perform controls even though much time had passed and the deck was effectively stacked against them. Methodologies such as this allow confident detection of genuine synesthetes because the surprise retest over lengthy intervals places the performance of synesthetes beyond the usual abilities of the average person. The drawback to this methodology, however, is that the task is extremely time-intensive to perform, and risks a high drop-out rate if synesthetes become untraceable at retest.

Perhaps for this reason, one of the most important developments in the methodology of synesthesia validation came with the introduction in 2007 of an alternative version of the test of genuineness. Eagleman and colleagues produced the *Synesthesia Battery*, a toolbox of online tests which provides a standardised set of questions, tests and quantitative scores to assess a range of synesthesias (Eagleman, Kagan, Nelson, Sagaram, & Sarma, 2007). This battery is again based on internal consistency in that synesthetes are validated by high consistency within their own synesthetic associations, stated repeatedly. However, consistency is measured within a single test session lasting only approximately 10 min. Specifically, synesthetes log on to the testing site (www.synesthete.org) and specify which form(s) of synesthesia they experience. The testing platform then presents their triggering stimuli (e.g., the 26 letters) one by one in randomized order, and participants are required to select their synesthetic colour for each trigger. Each stimulus (e.g., letter) is presented three times each, and a score is generated to quantify the consistency of participant's responses (e.g., did the participant choose the same/similar colours each of the three times she saw a particular letter?) This score represents the geometric distance in RGB (red, green, blue) colour space, where R, G, and B values are all normalised to lie between 0 and 1. If the mean overall score of colour-distance is less than 1, the participant is classified as a synesthete; if the score is 1 or higher, the degree of inconsistency classifies the participant as a non-synesthete. However, it remains an open question whether this limited retest interval is sufficient to truly distinguish synesthetes from non-synesthetes.

In the current study we assessed the validity of the Synesthesia Battery by using it to test almost 3000 randomly sampled subjects for grapheme-colour synesthesia. Our aim was to establish the prevalence of grapheme-colour synesthesia by this method. This will allow us to evaluate the Synesthesia Battery by comparing this prevalence – obtained by assessments within in a single test session – to the most widely accepted previous estimate of the prevalence of grapheme-colour synesthesia based on the standard *longitudinal* test–retest method (Simner, Mulvenna et al., 2006). If the Synesthesia Battery is just as effective a method for detecting synesthesia as the more standard long-term retest method, we anticipate an equivalent prevalence of grapheme-colour synesthesia across both methods. In carrying out our study, we chose to evaluate grapheme-colour synesthesia in particular for several reasons: it is one of the most common forms of synesthesia (Simner, Mulvenna et al., 2006), it is particularly well-understood in behavioural terms, it lends itself readily to online testing, and those who experience it typically demonstrate the high levels of consistency expected from synesthetes (compared to other variants, whose more complex concurrents may make them more difficult to assess via consistency alone; see Simner, Gäartner, & Taylor, 2011 for discussion). It was not our intention to change or try to improve upon the method made available by Eagleman et al. at www.synesthete.org. Rather, we attempted to simply replicate their test and methodology and then evaluate how it performs in comparison to a conventional longitudinal test–retest method.

In evaluating the Synesthesia Battery, our data will also provide an independent test of the prevalence of synesthesia. Our baseline study – the widely cited prevalence study of Simner, Mulvenna et al. (2006) – found the prevalence of grapheme-colour synesthesia to be 1.4% (for synesthetes with both coloured letters *and* numbers) or 2% (for synesthetes with either coloured letters *or* numbers). This study was based on a sample of 500 individuals, and the prevalence rate it generated was subsequently verified by a secondary method testing a further 1190 individuals (see Section 4 for details of this second method).

Two previous studies have also aimed to validate aspects of the Synesthesia Battery (Eagleman et al., 2007; Rothen, Seth, Witzel, & Ward, 2013). Both studies used self-reported synesthetes who had self-referred for study, in comparison to a group of controls declaring they were non-synesthetes. it is important to highlight the difference between *self-referred* and *self-reported* synesthetes. Self-reported synesthetes are any individuals who claim they have synesthesia. Self-referred synesthetes are those who have additionally made the effort to contact a university researcher to volunteer to take part in synesthesia studies. All synesthetes tested by Eagleman, Rothen and colleagues were not only self-declared, but also, importantly, self-referred. In comparison, none of the synesthetes tested here are self-referred. Instead, our approach is to screen the general population (some of whom at a certain point during our test, will self-report having synesthesia when asked, but will not be self-referred). There are likely to be significant differences between our own synesthetes, and the self-referred synesthetes of Eagleman, Rothen and colleagues. These latter synesthetes not only know they have coloured letters, but also know this is called synesthesia, and furthermore, they have made the effort to contact a university researcher to volunteer to take part in synesthesia studies. They therefore have an understanding of synesthesia and judge that the extent of their synesthesia is worthy of study by researchers. In other words, it is at least possible that self-referrers have relatively 'strong' (or noticeable, or attention-catching) synesthesia in some way and may not be entirely representative of the population of synesthetes at large.

In summary, because of the sampling methods of the two previous validations (Eagleman et al., 2007; Rothen et al., 2013) their participant groups (synesthetes versus controls) may have had diametrically opposing synesthesia characteristics, which might have therefore made them relatively easy to distinguish between. Indeed, both Eagleman et al. (2007) and Rothen et al. (2013) obtained a bimodal distribution of scores when assessing the consistency of grapheme-colour associations of their self-referred synesthetes compared to controls. Here however we individually assess a *randomly recruited* sample of subjects, allowing our own study to extend the previous findings of Eagleman et al. and Rothen et al. and establish how the Synesthete Battery performs when a distinction between synesthetes and non-synesthetes is perhaps more difficult to achieve. Put differently, by testing a random sample of the population, we expect to capture a broader, more representative range of synesthetic experiences, and we are evaluating how the Synesthete Battery performs under these conditions. In addition, our study will provide the largest estimate of the prevalence of grapheme-colour synesthetes to date, with almost 3000 randomly sampled members of the general population.[1]

Finally, our study also investigates a second aspect of the Synesthesia Battery. After the single session test of consistency of coloured graphemes, participants next immediately perform a second test of synesthesia: a *speeded congruency verification task* (Eagleman et al., 2007). In this, participants are presented with individual graphemes, but this time the graphemes are coloured either to match the participant's earlier colour selection (congruent), or to be a different colour (incongruent). Participants must simply answer whether the letter-colour pairing matched their previous choices or not, and their accuracy and speed is measured. Eagleman et al. (2007) report that synesthetes tend to score 90% or higher with a mean RT of 0.64 ± 0.78 s, while non-synesthetes score below 90% with mean RT of 0.91 ± 0.87 s (Eagleman et al., 2007). We will examine the sensitivity of this type of test to determine whether it too has the diagnostic capability to distinguish between participants who score below the consistency threshold score of <1 (i.e., synesthetes) and those who do not. In other words, where Eagleman et al. (2007) compared self-referred synesthetes with non-synesthete controls, we will extend this type of test to assess (a) randomly sampled participants, who are (b) self-declared synesthetes, but who are not self-referred synesthetes, and who are also (c) either verified as genuine versus non-genuine. As such, we are evaluating whether this type of speed-congruency test still holds up in what is likely to be a more sensitive comparison.

2. Methods

2.1. Participants

Two thousand eight hundred and forty-seven participants took part in our study (1317 male, 1530 female; mean age 28.6, range 16–90, S.D. 14.3). We had additionally tested 32 further subjects who completed our study but had entered an

[1] Early estimates of prevalence in the science literature had varied widely, at least partly because researchers were focussing on different sub-types or using different definitional criteria (Ramachandran & Hubbard, 2001). Even in studies that aimed to report the prevalence of all forms, estimates range from 1 in 4 (Calkins, 1895; Domino, 1989; Uhlich, 1957), to 1 in 10 (Rose, 1909), 1 in 20 (Galton, 1883), 1 in 200 (Ramachandran & Hubbard, 2001), 1 in 2000 (Baron-Cohen et al., 1996), and 1 in 25 000–100 000 (Cytowic, 1993, 1997). A number of these studies were simply 'best guesses', while others failed to use objective tests to verify synesthetic reports (e.g., Calkins, 1895; Domino, 1989; Rose, 1909; Uhlich, 1957). Others still did use robust consistency methods to verify synesthesia, but were subject to a recruitment bias: their subjects were recruited in response to adverts in newspapers, leaving open the possibility that low prevalence rates (e.g., 0.05–1% of the population; Barnett, Finucane, Asher, Bargary, Corvin, Newell, & Mitchell, 2008; Baron-Cohen et al., 1996) were due simply to poor rates of responding (see Baron-Cohen et al., 1996 for discussion). The estimate we use here (from Simner, Mulvenna et al., 2006) is a particularly robust estimate since it avoided the problems of self-referral by individually questioning every member of a participant pool, and using an objective test of genuineness (consistency over long-term retest).

obviously false date of birth (e.g., 2013). These subjects did not enter our analysis, which was therefore based only on our $N = 2847$.

Participants were recruited as part of a large-scale, centrally co-ordinated undergraduate research project. Every student registered on the 2nd year of the Psychology undergraduate course at the University of Edinburgh acted as a research assistant (RA), and was required to each recruit 8 participants (4 male and 4 female) over 16 years of age. Our student RAs were not allowed to take part in the study themselves. In recruiting our participants, we took a number of steps to ensure as random a sample as possible. First, RAs were instructed not to deliberately seek out, nor to avoid, people they knew to be synesthetes. Furthermore, in order to avoid self-referral biases, RAs were required to pre-select their sample, and then approach participants in a targeted way (rather than send out an advert and accept self-referrals). Indeed, RAs were required to refrain from recruiting participants via any open calls at all, for example, they could not post the testing URL on social media websites or internet forums. Finally, RAs were also instructed not to *a priori* inform participants that the study involved synesthesia. The instructions given by the RAs to prospective participants were uniform, and clearly stated that participants were only allowed to complete the test once; if they had previously been approached to complete the test by someone else, they were to inform the recruiter and not proceed with the test.

Our study was carried out in two waves to maximise participation numbers: 1514 were tested in January 2013, and 1333 were tested in September 2013. Both used identical methods, carried out by two consecutive intakes of 2nd year students. In both rounds, the study was carefully managed and co-ordinated by authors JS and DAC. Data from both rounds are pooled and presented together here.

2.2. The online test

The online test consisted of several sections. Participants first provided informed consent via a checkbox and then gave demographic information such as age, sex, handedness and native language. A second section consisted of a health questionnaire not relevant for the current study. (In this, subjects were requested to indicate if they suffered from a range of clinical conditions, and this was for another project to be reported elsewhere.) After this page, our online synesthesia assessment began with our locally stored replica of the Synesthesia Battery. In this replica – as in the original – participants were first asked whether they experienced grapheme-colour synesthesia, with the question "Do numbers or letters cause you to have a colour experience?" This was accompanied by an example, and then an option to accept separately according to whether these colours are triggered automatically by numbers and/or digits. If participants indicated that they saw neither letters nor numbers in colour, they advanced to an early-exit page thanking them for their participation.

The rest of the test was completed by participants who answered in the affirmative to having coloured letters/digits. These participants completed two further tests tailored to the particular variant of grapheme-colour synesthesia they had reported (i.e., for either digits, letters, or both). These two tests were a colour consistency test and a speeded congruency task. The colour consistency test was again an identical clone of the consistency test from the Synesthesia Battery (Eagleman et al., 2007). In this, participants were presented with each grapheme (a–z, 0–9) three times in random order (so 30 trials if the subject reported coloured numbers only, 78 trials if letters only, and 108 trials if both letters and numbers). For each trial, participants were required to select the colour that best matched the grapheme presented (see Fig. 1). Selections were made from a palette of $256 \times 256 \times 256$ colours, exactly as in the original Synesthesia Battery. Once their selection was submitted, the screen advanced to show the next grapheme. The colour palette followed an HSL colour model, with colours varying in lightness along the vertical axis and saturation along the horizontal access a separate, horizontal bar allowed hue to be adjusted (see Fig. 1).

The colour consistency test was followed by a speeded congruency task. In this section, participants were shown again the graphemes they had just seen in the colour consistency test. This time they saw each relevant grapheme twice, in a random order, each flashed on screen for a maximum of 1 s or until the participant responded (20 trials for just numbers, 52 trials for just letters or 72 trials for both letters and numbers). In 50% of trials, graphemes were coloured congruently with the participant's earlier specification, and in 50% of trials they were coloured incongruently. Participants were required to indicate by mouse-click on the relevant on-screen button whether the each grapheme they saw either matched or did not match their previous colour pairing (as collected during the consistency test; Fig. 2). Their response mouse-click advanced the test to the next grapheme, and the test continued until all graphemes had been shown.[2]

[2] On completing our study we discovered minor methodological differences to Eagleman et al. (2007) in how congruent and incongruently coloured graphemes were selected and presented. In our study here, the first presentation of graphemes included 50% that were randomly selected to be presented in in their congruent colour, taken from the participant's 1st selection in the prior task. For the second presentation, 50% of graphemes were again randomly selected to be congruently coloured, this time from the participant's 2nd selection from the previous task. The incongruent colours were randomly selected in RGB space, and were not taken from the participants' earlier palette. In Eagleman et al. (2007), each grapheme was presented twice in a fully randomised order – once congruently (always from the 1st selection given previously) and once incongruently. The incongruent colour was chosen from the participant's earlier palette of colours on the consistency test and verified to be sufficiently distant in RGB space from the congruent colour. The details of this were not given in the Eagleman paper. Furthermore, in our own study a response press advanced the display onto the next grapheme, while in Eagleman et al. (2007) the grapheme was displayed for a constant 1s followed by a fixed inter-stimulus interval. These differences mean we can no longer directly compare our data in this section to that from Eagleman et al. (2007). Instead, our investigation here will serve to determine whether this *type* of study (i.e., a speeded congruency task) can be validated on our new participant population and how responses in this type of test might relate to the consistency scores provided by the Synesthesia Battery. Finally we point out that our central aim – to assess the consistency portion of the Synesthesia Battery – is unaffected.

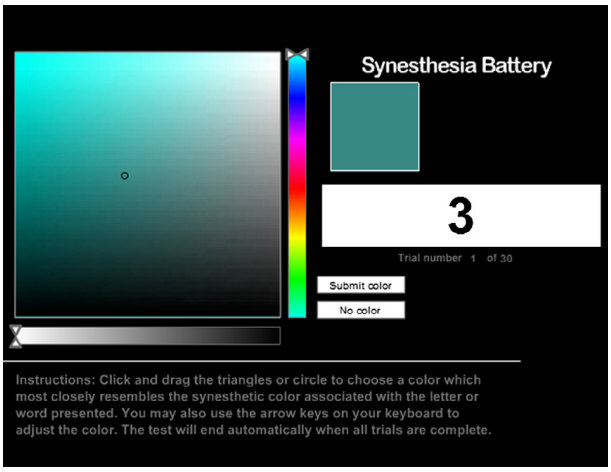

Fig. 1. Screenshot from the consistency test.

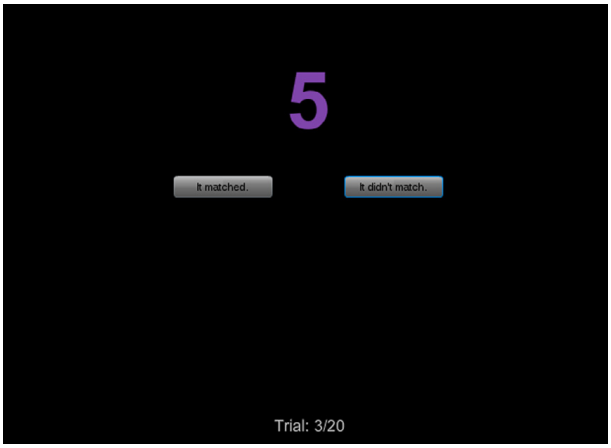

Fig. 2. Screenshot from the speeded congruency task.

In summary, the colour consistency test generated a consistency colour-distance score, and the speeded congruency task generated an accuracy score and a reaction time. For full details of website configuration and how the consistency colour-distance score is calculated, see Eagleman et al. (2007).

3. Results

In our study, we classified as non-synesthetes all those who were directed to the early-exit page (i.e., those who said they did not experience coloured letters and/or digits) and all those who continued but scored 1 or higher. The remainder were classified as synesthetes (i.e., those who scored <1).

From our sample of 2847 participants, 140 subjects (55 male, 85 female, mean age 23.9, range 16–71, SD 9.5) self-reported grapheme-colour synesthesia, giving a self-reported prevalence of 4.9%. Of those 140 self-reported synesthetes, 34 obtained a colour-distance score of <1 on their consistency test (14 male, 20 female, mean age 24.9, range 17–51, SD 5.8), which is the criterion used by Eagleman et al. (2007) to identify genuine synesthesia. This places the prevalence of genuine grapheme-colour synesthesia at 1.2% and we will return to this prevalence value further below.

Of the 140 self-reported synesthetes, 55 reported experiencing coloured numbers only, 58 reported experiencing both coloured numbers and letters and 27 subjects reported experiencing coloured letters only (see Fig. 3a). Of the 34 participants that scored <1 on the consistency test, 17 experienced coloured numbers only, 14 experienced both coloured numbers and letters and 3 subjects experienced coloured letters only (see Fig. 3b). As an additional check, the colour choices of the participants obtaining a consistency score of <1 were examined individually to allow us to confirm that none of these achieved their superior consistency by entering the same colour for each grapheme, or by entering an obviously non-synesthetic pattern of colours throughout, e.g. red for 'R', green for 'G' and blue for 'B' (following Simner, Mulvenna et al., 2006).

Next we analysed accuracy and RTs in the speeded congruency task. We first divided our sample of self-reported synesthetes into two groups, around to the consistency colour-distance threshold of <1. For clarity, we refer to those who scored

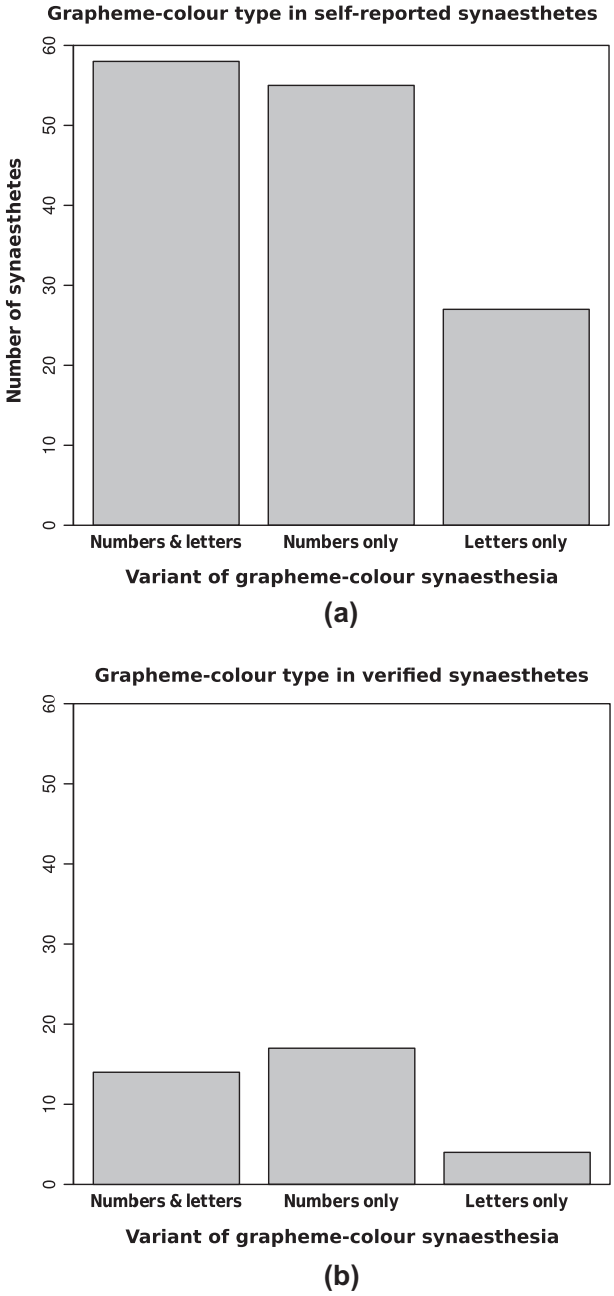

Fig. 3. Variants of grapheme-colour synesthesia reported by (a) all 140 participants who self-reported synesthesia, and (b) the 34 participants confirmed as genuine synesthetes (i.e., with colour-distance consistency scores of <1).

<1 as *genuine synesthetes*, and those who score $\geqslant 1$ we refer to as *malingerers*[3] (i.e., non-synesthetes who self-reported synesthesia but failed to achieve what is considered a synesthetic score in the colour consistency test). There were 34 genuine synesthetes, as noted above, and 90 malingerers. There were also 16 subjects who reported too few coloured graphemes to generate a consistency colour-distance score at all. Following Eagleman et al., 2007, participants were required to enter a minimum of two valid graphemes to obtain a consistency score, a valid grapheme being defined as one to which the subject entered a colour for all three presentations. These 16 are omitted from all further analyses below. (Finally, we remind the reader that there are no values for participants who declared themselves to be non-synesthetes from the start because these individuals did not progress to the synesthesia assessment.)

[3] We use this term only for clarity but are in fact agnostic about the nature of these individuals. The prevailing view is that these are likely to be non-synesthetes falsely reporting synesthesia and therefore failing the objective consistency test. However, we leave open the possibility that some genuine synesthetes may not in fact be consistent over time, and refer to the reader to Simner, Mulvenna et al. (2006), Simner (2012), Eagleman (2012) and Cohen Kadosh and Terhune (2012) for further discussion on this matter.

Using an independent samples *t*-test, we calculated the mean accuracy in the speeded congruency task for each group: genuine synesthetes versus malingerers. This mean was 84.4% (SD = 11.8) for synesthetes and 70.5% (SD = 14.9) for malingerers, and this difference was significant ($t(122) = 5.45$, $p < .0001$, $d = 1.03$). There was no significant difference in mean reaction times between groups (Synesthetes $M = 1.93$ s; SD = 0.77; Malingerers $M = 1.76$ s, SD = 0.73; $t(122) = 1.13$, $p = .13$, $d = 0.23$) see Fig. 4a and b). Mean reaction time was calculated across all trials, irrespective of whether the participant had answered correctly or incorrectly.[4]

In order to investigate whether this non-significant result provided evidence for the null hypothesis we calculated a Bayes factor. By comparing the likelihood of two models (in this case, the null and alternative hypotheses) as a ratio, Bayes factors allow the researcher to evaluate to what extent the data supports the null hypothesis (Rouder, Speckman, Sun, Morey, & Iverson, 2009). Following Jeffreys (1961), a Bayes factor of less than 0.33 provides strong support for the null hypothesis, a Bayes factor of greater than 3 provides support for the alternative hypothesis and values inbetween indicate the data are insensitive and no firm conclusions should be drawn. Using the online calculator provided by Rouder et al. (2009), we calculated a Bayes factor of 1.17, indicating that the data are not sensitive enough to enable a conclusion to be drawn.

Finally we explored how these two types of test (consistency and speeded congruency) work in tandem in their assessments of synesthesia. If we take self-reported synesthetes as a single group (i.e., collapsing genuine synesthetes and malingerers) there was a significant inverse correlation between the consistency colour-distance score, and the speeded congruency accuracy score ($r(122) = -.71$, $p < .001$; see Fig. 5). Remembering that low colour-distance scores and high accuracy scores are both indicative of synesthesia, this inverse correlation shows that those who performed like synesthetes in the first sub-test were also more likely to perform like synesthetes in the second. However, when we calculate this correlation for genuine synesthetes and malingerers separately, it becomes apparent that the effect comes from the malingerer group only. The correlation between consistency colour distance score and speeded-congruency accuracy score for genuine synesthetes is non-significant ($r(32) = -.07$, $p = .35$) whereas for the malingerers, the correlation is highly significant ($r(88) = -.73$, $p < .0001$).

Finally, there was no significant correlation between consistency colour-distance score and reaction time ($r(122) = -.05$, $p = .59$), nor for accuracy score and reaction time ($r(122) = -.11$, $p = .22$), and this also remained the case when correlations for the synesthete and malingerer groups were calculated separately (synesthete consistency-RT = $r(32) = .006$, $p = .49$; malinger consistency-RT = $r(88) = .014$, $p = .45$) (synesthete accuracy-RT = $r(32) = -.18$, $p = .14$; malinger accuracy-RT = $r(88) = -.09$, $p = .19$).[5]

3.1. Prevalence comparison between studies: Comparing short versus long-term testing

The key aim of this study was to compare the prevalence of grapheme-colour synesthesia generated by the Synesthesia Battery (a single-session test) to a more conventional method based on long term (rather than single session) testing (Simner, Mulvenna et al., 2006). Using the Synesthesia Battery we found the prevalence of grapheme-colour synesthesia in the general population to be 1.2% for those with coloured letters *or* digits, compared to the previous estimate of 2% in conventional longer-term testing (Simner, Mulvenna et al., 2006). We can now evaluate that these two estimates are not significantly different (chi square = 2.1; $df = 1$; $p = .14$). However, we also calculated prevalence for synesthetes with both coloured letters *and* digits, finding a value here of 0.5%, and this is significantly less than the 1.4% found in conventional longer term retesting (Simner, Mulvenna et al., 2006; chi square = 5.6; $df = 1$; $p = .02$).

4. Discussion

In our study, we reproduced elements of the Synesthesia Battery (Eagleman et al., 2007) which is a single-session online test for grapheme-colour synesthesia. We used this method to estimate the prevalence of grapheme-colour synesthesia in a very large randomly recruited sample – indeed the largest sample for this purpose to date. We found the prevalence of grapheme-colour synesthesia in the general population to be 1.2% for those with coloured letters *or* digits, compared to the previous estimate of 2% (Simner, Mulvenna et al., 2006), a difference which is not significantly different. However, we also calculated prevalence of synesthetes with both coloured letters *and* digits, finding a value here of 0.5%, and this is significantly less than the 1.4% found in conventional longer term retesting (Simner, Mulvenna et al., 2006). Hence, the Synesthesia Battery numerically under-estimated the prevalence of those with coloured letters and digits, compared to longer retesting methods and we explore possible reasons for this below.

One explanation for an under-estimation of prevalence in the Synesthesia Battery might stem from the way graphemes are presented to those who report more than one variant: letters and digits are presented together, randomly ordered within

[4] We analysed both correct and incorrect trails was because our aim in this paper was to replicate Eagleman et al.'s (2007) methods as faithfully as possible. However, when analysing reaction times in Stroop-like behavioural tasks, convention typically dictates that only correct trials are included in the analysis. When only correct trials are analysed, the result does not change: there is no significant difference in reaction time between genuine synesthetes and malingerers ($p = .95$), which – like before – remains contrary to Eagleman et al.'s original finding.

[5] An anonymous reviewer has asked us to comment on the age of our subjects. The age range of all participants was 16–90 years. No genuine synesthetes were found in the age range 52–90. Four participants in this age range reported having synesthesia but scored >1 in the colour distance consistency test, so were classified as malingerers.

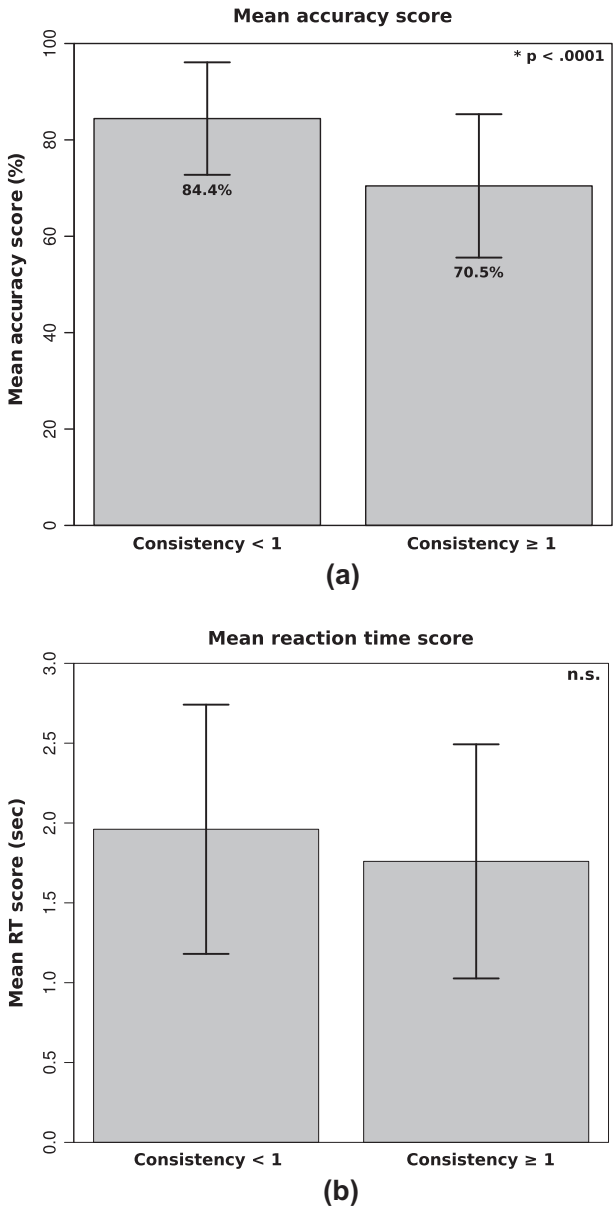

Fig. 4. Results from the speeded congruency task showing that (a) genuine synesthetes (indicated as "consistency <1") were significantly more accurate than malingerers (indicated as "consistency ⩾1") and that (b) there was no significant difference in mean reaction time between these two groups. (Note that there are no values for participants who declared themselves to be non-synesthetes from the start because these individuals did not progress to the synesthesia assessment.)

the same consistency test. In the longer term retesting used as the baseline here however, letters and digits were always presented in separate blocks (Simner, Mulvenna et al., 2006). It may therefore be that synesthetes are susceptible to interference when selecting colours for graphemes, and we raise this possibility for future studies to consider. A second possible explanation for the lower prevalence found here is that immediate retests might be inherently more conservative, and this might be suggested by the fact that prevalence estimates here were numerically lower across the board. Immediate retesting might be conservative because the scores of *non-synaethetes* are likely to be better across shorter (versus longer) time intervals. In other words, non-synesthetes in an immediate retest (e.g., over 10 min) should score higher than in a delayed retest (e.g., over several weeks) even though synesthetes' scores, in comparison, would be likely to remain relatively unchanged. This would raise the control baseline for single session testing, and therefore allow a smaller range of synesthetes to be identified as significantly more consistent. If this is correct, we conclude that the threshold at which synesthetes are identified (currently around the score of 1) might be usefully set marginally higher in the Synesthesia Battery of Eagleman et al. (2007).

One recent study has also speculated that the threshold in the Synesthesia Battery perhaps should be raised. Rothen et al. (2013) have recently argued that for the combination of RGB colour space and city block distance used by Eagleman et al. (2007) – and also in this study – a higher cut-off may indeed be more appropriate. In order to maximise sensitivity and specificity for this colour space/distance combination, they proposed a revised threshold within the existing Synesthesia Battery

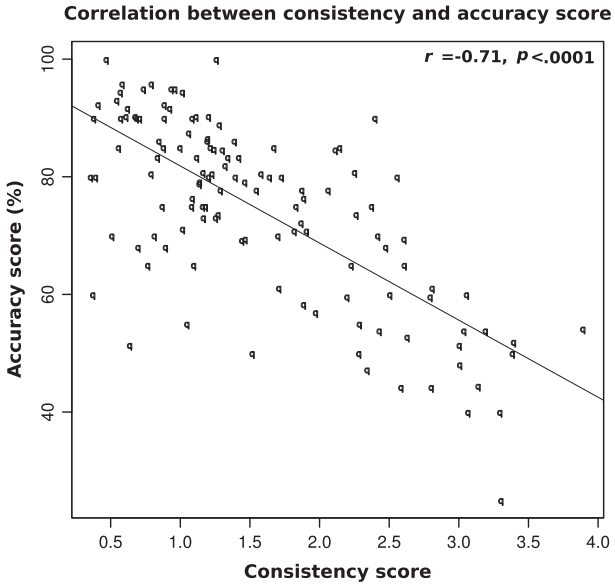

Fig. 5. Scatterplot showing a significant association between colour-distance consistency score and accuracy score for self-reported synesthetes.

at <1.43 for synesthetes (rather than <1). In our own study, if we recalculate prevalence in our sample using Rothen et al's suggested cut-off score of 1.43, our prevalence estimates are no longer significantly lower than expected. With this revised threshold, we now find 69 genuine synesthetes with coloured letters OR digits (2.4%; compared to 2% in Simner, Mulvenna et al., 2006; chi square = 0.3; $df = 1$; $p = .57$) and 31 synesthetes with coloured letters AND digits (1.1%; compared to 1.4% in Simner, Mulvenna et al., 2006; chi square = 0.367; $df = 1$; $p = .5$). In other words, our results are more in line with more conventional longer term evaluations of synesthesia when the threshold is shifted upwards according to the proposal of Rothen et al. (2013).[6] Finally, we point out that Rothen and colleagues also suggest that using alternative colour models (CIELUV and CIELAB) and Euclidean distance provide the best combination of sensitivity and specificity in distinguishing between synesthetes and non-synesthetes. However that change would be beyond the scope of this paper, where we are simply evaluating the Eagleman data output as it stands.

Our discussion above of where to set the cut-off threshold for consistency in the Synesthesia Battery might be considered part of a more fundamental concern in synesthesia research. In all kinds of consistency tests for synesthesia – and particularly obvious here – participants are divided into two groups by their score on what is in fact an incremental continuum of possible scores. (Using this test, participants can, in theory, score any value between 0 and ~4, even though the cut-off is conventionally placed at the fixed value of 1). It seems clear that someone who scores 1.05 on the consistency test is "more synesthetic" than someone scoring 2.48, yet according to the cut-off of <1, both would be considered non-synesthetes. Nonetheless, it is a particular strength of the Synesthesia Battery that researchers are free to consider this score in its own right, rather than for categorical groupings alone.

The second part of Eagleman et al's test involved speeded congruency task in which graphemes are presented either in the same colour previously selected (congruent) or a different colour (incongruent). Subjects must indicate whether the colour they saw matched their earlier choice, and Eagleman et al. (2007) report significant differences in speed and accuracy between a pre-selected group of 15 self-referred synesthetes, and a non-synesthete control group. In our current study, when our randomly-sampled respondents were divided into genuine synesthetes versus malingerers (i.e., around the threshold score of 1), genuine synesthetes were again significantly more accurate than malingerers. These data suggest that accuracy scores can not only distinguish between self-referred synesthetes and non-synesthetes (as in Eagleman et al., 2007) but is also subtle enough to distinguish between groups of genuine synesthetes and those who claim to be so, but do not pass a conventional consistency test.

Furthermore, considering all self-reported synesthetes irrespective of their consistency, there was a significant inverse correlation between consistency and accuracy, indicating that more consistent (i.e., more 'genuine') synesthetes were also more accurate. However, when this correlation was calculated separately for the each group (genuine synesthetes and malingers) it became apparent that the effect was driven by the malingerers only. We suggest this is because genuine synesthetes score highly on the accuracy test, irrespective of what consistency score they achieve. In other words, a synesthete obtaining a consistency score of, say, 0.99 is likely to be highly accurate on the speeded-congruency test – as accurate as

[6] If we repeat our earlier analyses from the speeded-congruency test (looking at differences in mean accuracy score and reaction time) grouping synesthetes and non-synesthetes according to the revised threshold of <1.43, neither result changes. Synesthetes remain significantly more accurate than non-synesthetes (82.8% (SD = 10.3) versus 63.7% (SD = 14.3); ($t(95.27) = 8.32$, $p < .0001$, $d = 1.53$). There remains no significant difference in reaction times between synesthetes and non-synesthetes (1.71 s (SD = 0.56) versus 1.58 s (SD = 0.81); ($t(92.04) = 0.97$, $p < .17$, $d = 0.19$).

a synesthete scoring 0.4 on the consistency test. In contrast, a malingerer obtaining a consistency score of 1.5 is more likely to be more accurate on the speeded-congruency test than a malingerer scoring 3.5 for consistency.

The speed accuracy congruency test presented here showed one notable difference in results compared to Eagleman et al. (2007). Our own findings were that genuine synesthetes, although more accurate than malingerers, were no faster. Eagleman and colleagues found genuine synesthetes to be more accurate *and* faster than controls. This difference to Eagleman et al. (2007) may stem from differences in our control populations: Eagleman et al. (2007) compared synesthetes to self-declared non-synesthetes, while we compared to 'malingerer' individuals claiming to have synesthesia. It is possible that some portion of our controls were in fact synesthetic in some way, albeit with lower consistency, and perhaps this is why our genuine synesthetes did not differ from them in their RTs (see above and Simner, 2012 for discussion). Alternatively, our lack of difference in RTs may be the result of the unanticipated variations we introduced in our version of this test. Our method of selecting congruent and incongruent graphemes and the timing of grapheme presentation for this part of the test differed slightly from Eagleman et al.'s original approach (see Section 2 for a full explanation). Our own version may have raised the difficulty of the task (e.g., because graphemes were on-screen for a shorter time on average) and our RTs were certainly longer and hence potentially more noisy. There is no way to distinguish between these two hypotheses in the current study and so we leave this as an open question for future studies to address.

We have evaluated whether single session tests of consistency are effective at identifying synesthetes, compared to established longer retesting methods. One previous study has also suggested that single session testing may indeed be valid. Simner, Mulvenna et al. (2006) established the prevalence of synesthesia both with long term testing (which we used here as our key comparison) but also by screening additional 1190 people in a single session. Their method was more basic than that of Eagleman et al. (2007) in that colour choices were made from a palette of just 13 colours, and only absolute matches contributed to consistency scores. Nonetheless, this again produced roughly comparable prevalence estimates as longer term testing (1.1% prevalence for coloured letters and digits). Taken together with the current study, we therefore suggest single session tests of consistency for synesthetic associations do appear to provide an appropriate method by which to identify synesthetes. The widely available nature of the Synesthesia Battery through its open-access online interface makes it a particularly appealing version of this test, as does its comprehensive colour palette, and its ability to give a calibrated estimate (i.e., continuous consistency score) for synesthesia status. Although researchers will want to consider carefully the question of whether consistency testing can reliably identify every type of synesthesia, or indeed every type of synesthete (see Simner, 2012 for discussion), it is clear from our current study that the Synesthesia Battery provides a suitable tool for evaluating synesthetes along this dimension.

Acknowledgments

Correspondence and requests for materials should be addressed to DAC (e-mail: d.a.carmichael@ed.ac.uk). We are grateful to Marco Elver for assistance with database configuration and setup. DAC was supported in part by grants EP/F500385/1 and BB/F529254/1 for the University of Edinburgh, School of Informatics Doctoral Training Centre in Neuroinformatics and Computational Neuroscience (www.anc.ac.uk/dtc) from the UK Engineering and Physical Sciences Research Council (EPSRC), UK Biotechnology and Biological Sciences Research Council (BBSRC), and the UK Medical Research Council (MRC). Author JS was supported in part by the European Research Council under the European Union's Seventh Framework Programme (FP/2007-2013) / ERC Grant Agreement n. [617678].

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Appendix H

Colour fluctuations in grapheme-colour synaesthesia: The effect of clinical and nonclinical mood changes

British Journal of Psychology (2014)

The British Psychological Society

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Colour fluctuations in grapheme-colour synaesthesia: The effect of clinical and non-clinical mood changes

Collette L. Kay[1], Duncan A. Carmichael[1], Henry E. Ruffell[1] and Julia Simner[1,2]*

[1]Department of Psychology, University of Edinburgh, UK
[2]School of Psychology, Pevensey Building, University of Sussex, Falmer, UK

Synaesthesia is a condition that gives rise to unusual secondary sensations (e.g., colours are perceived when listening to music). These unusual sensations tend to be reported as being stable throughout adulthood (e.g., Simner & Logie, 2007, *Neurocase*, 13, 358) and the consistency of these experiences over time is taken as the behavioural hallmark of genuineness. Our study looked at the influence of mood states on synaesthetic colours. In Experiment 1, we recruited grapheme-colour synaesthetes (who experience colours from letters/digits) and elicited their synaesthetic colours, as well as their mood and depression states, in two different testing sessions. In each session, participants completed the PANAS-X (Watson & Clark, 1999) and the BDI-II (Beck, Steer, & Brown, 1996, Manual for Beck Depression Inventory-II), and chose their synaesthetic colours for letters A-Z from an interactive colour palette. We found that negative mood significantly decreased the luminance of synaesthetic colours. In Experiment 2, we showed that synaesthetic colours were also less luminant for synaesthetes with anxiety disorder, versus those without. Additional evidence suggests that colour saturation, too, may inversely correlate with depressive symptoms. These results show that fluctuations in mood within both a normal and clinical range influence synaesthetic colours over time. This has implications for our understanding about the longitudinal stability of synaesthetic experiences, and of how mood may interact with the visual (imagery) systems.

Synaesthesia is an inherited condition that causes unusual secondary sensations. For example, synaesthetes may see colours when smelling odours (Day, 2013), or they might experience tastes when hearing words (Ward & Simner, 2003) or listening to musical notes (Beeli, Esslen, & Jäncke, 2005). These experiences feel intrinsically normal to the synaesthete, who will have experienced them since childhood (at least in the case of *developmental* synaesthesia, the focus of the current article). Synaesthesia is found in at least 4% of the population (Simner *et al.*, 2006), and two recent genome-wide studies have identified genetic regions of interest on chromosomes 2, 5, 6, and 12 and 16 (Asher *et al.*, 2009; Tomson *et al.*, 2011). Synaesthesia is a multi-phenotypic condition depending on which modalities are merged (e.g., sound with colour, taste with shape etc.), but one of the most common forms is *grapheme-colour synaesthesia* (Simner *et al.*, 2006) in which

*Correspondence should be addressed to Julia Simner, School of Psychology, Pevensey Building, University of Sussex, Falmer BN1 9QH, UK (email: j.simner@sussex.ac.uk).

DOI:10.1111/bjop.12102

colour experiences are triggered by reading, hearing or thinking about graphemes (letters or numbers). In scientific parlance, (Grossenbacher & Lovelace, 2001) graphemes would be the 'inducer' stimulus in this variant, and colour would be the 'concurrent', and we shall use this terminology throughout our article. In this article, we will look at how synaesthetic concurrents of colour can be influenced by the mood states of synaesthetes.

Since one person cannot access another's perceptual experiences, synaesthesia researchers rely on measuring the behaviour of synaesthetes, or their neurophysiological responses, but they also elicit first person accounts. When adult synaesthetes report their concurrent sensations for any given list of inducers, these tend to be highly consistent over time. In other words, a given inducer (e.g., the letter A) tends to elicit the same synaesthetic concurrent (e.g., the colour red) for any given synaesthete in repeated testing. Indeed, synaesthetic sensations are often thought to be consistent *by definition* throughout the adult lifespan and this consistency-over-time is taken as the behavioural hallmark of synaesthesia in genuineness testing (see Johnson, Allison, & Baron-Cohen, 2013 for review, and Simner & Bain, 2013 for how this constancy develops in childhood). In a typical 'genuineness test' of synaesthesia (e.g., Baron-Cohen, Wyke, & Binnie, 1987), synaesthetes are required to report their synaesthetic associations for a stimulus list (e.g., a list of letters), and must be significantly more consistent in a retest compared to a group of non-synaesthete controls, who are asked to invent analogous associations (i.e., to make-up colours for the 26 letters and then recall these later). Synaesthetes tend to be highly consistent and have been shown to out-perform controls even when tested over far longer time intervals, and even when controls are given monetary incentives to perform well (e.g., Ward, Simner, & Auyeung, 2005). For example, synaesthete EP was 100% consistent in her colours for a set of words across 1 year, compared to a non-synaesthete control who was just 17% consistent over 2 weeks (Baron-Cohen *et al.*, 1987).

Research into synaesthesia has relied heavily upon this consistency feature because it is the standard by which synaesthetic status is traditionally verified. But although virtually every synaesthete described in the literature has been shown to be significantly more consistent than controls, they are not necessarily 100% consistent. Grapheme-colour synaesthetes in a study by Simner *et al.* (2006), for example, had consistency scores that ranged from 73% to 100%. All were considered synaesthetes because they were each significantly more consistent than controls. Nonetheless, this variation in consistency shows there can be a degree of impermanence in synaesthetic sensations, which is rarely talked about (but see Simner, 2012; Cohen Kadosh & Terhune, 2012; Eagleman, 2012). In this study, we ask what might account for variations in reported synaesthetic colours, and we begin by briefly exploring several theoretical possibilities below.

We first point out that inconsistently reported synaesthetic associations might be due to difficulties in *reporting*, rather than to inconsistency in the experience itself. For example, a synaesthetic colour that lies on the border between the two colour categories of, say, red and brown (i.e., a maroon-like colour) might be described as red on one occasion but brown on another, making the association appear inconsistent when it is in fact not. Alternatively, it might be difficult for the synaesthete to identify the sensation at all. Lexical-gustatory synaesthete JIW, for example, (who experiences floods of taste in the mouth when reading words) experiences tastes he cannot always identify, and this too can lead to reporting discrepancies (e.g., one taste was reported as Sugar Puffs© breakfast cereal on one occasion but Rice Krispies© breakfast cereal on another – even though the taste itself had not changed; Ward & Simner, 2003). Inconsistencies in reporting might also arise when a single inducer has more than one concurrent: grapheme-colour synaesthete JM, for example, reports that her letter L is 'both black and yellow. Not a mix

of those two, it's just both simultaneously' (personal communication). Again this might be artificially lower consistency scores, if JM chooses to report yellow on one occasion but black on another. Indeed, some synaesthetic concurrents are so highly complex that it is almost inevitable for inconsistencies in reporting to arise. Consider synaesthete MJS, for example, who has *sequence-personality synaesthesia* (in which letters, numbers and other sequences are synaesthetically imbued with personality qualities; Simner, Gärtner, & Taylor, 2011). Since personality has many facets, personality concurrents are necessarily complex, and this can cause misleading reports. MJS for example first described the number 2 as 'someone who gets things done', but later as 'a quiet type' (Simner *et al.*, 2011). This appeared inconsistent until she was asked again 4 years later, at which point she described the personality more fully as a 'good quiet little sort, can be counted on. Deals with it. Ideal employee' (Simner *et al.*, 2011; p. 293). Only then was it clear that the concurrent itself never changed, even though selective reporting had made it appear inconsistent. Finally, it is also possible that different facets of a concurrent are more prominent at different times.

In all instances above, the nature of the synaesthetic concurrent was unchanged over time but simply reported differently. It is possible, however, that concurrents themselves do change over time. This issue has been almost entirely overlooked in the literature and is poorly understood. In this article, we ask how synaesthetic colours might change from day-to-day, particularly under the influence of mood. Human moods or emotional states change across points in time, and it is therefore reasonable to ask whether these mood changes influence synaesthesia in some way. There are several reasons to believe they might. First, mood states can affect cognition, reasoning and attention (Blanchette & Leese, 2011; Compton, 2003; Oaksford, Morris, Grainger, & Williams, 1996; Pessoa, 2008; Wadlinger & Isaacowitz, 2006) so may indirectly influence synaesthesia simply because attention on the inducer, for example, is necessary for synaesthetic colours to be triggered (for review of attentional influences on synaesthesia, see Rich & Mattingley, 2013). Alternatively, changes in mood might influence synaesthetic experiences more directly, and if this is true, it could cause fairly regular fluctuations in synaesthetic experiences. If mood state does influence synaesthetic concurrents, we ask what form this influence might take.

To answer this, let us briefly review what is known about how mood and colour are related, both in synaesthetes and non-synaesthetes. In synaesthesia, there have been a small number of case reports of emotion as the direct inducer for a synaesthetic experience. One historical account describes case E, for whom 'experiences are emotionally coloured in a literal sense' (Cutsforth, 1925, p. 529). This descriptive account suggests that E experienced a range of stimuli (odours, sounds, memories) in terms of the colour of their emotional valence: pleasant stimuli were often blues, reds, and yellows, while unpleasant stimuli were often greens and browns. In more detailed account in the modern literature, Ward (2004) described GW, who reports synaesthetic colours on the presentation of emotionally valent words. When presented with a list of names for example, she experienced colours more for the names of people she knew (and therefore had emotional connections with) than of people she did not know. Common nouns, too, triggered colour according to their emotional value: words that were highly emotive such as 'love' elicited synaesthetic colours more often than neutral stimuli, such as 'chair'. There was also an association between emotions and the *nature* of the synaesthetic colour: positive emotive words had a lighter and more saturated colour, and more negative words were darker and less saturated. Ward (2004) therefore concluded that GW's inducer had an emotional component distinct from lexical qualities of the words

themselves, and that this linked high luminance and saturation to positive emotional valence.

Mood and emotional state are also associated with colour qualities for even the general population. Studies have shown that different colour qualities (i.e., of hue, saturation and luminance) are linked to different mood states. Manav (2007) found that subjects associated the colour yellow, for example, with more negative emotional adjectives when its luminance was lower (e.g., *boring*, *anxious*, *depressive*). Children too are already able to express coherent shared emotional responses to colours at the age of 5–6 years. When asked 'How does this colour make you feel?' they tend to give positive emotions to bright colours (e.g., pink, blue, red) and more negative emotions to dark or achromatic colours (e.g., brown, black, grey; Boyatzis & Varghese, 1994). A similar finding has been replicated in the adult population (Hemphill, 1996).

One final set of studies suggests again that mood variances may influence colour associations – this time for clinical mood states – and they can even influence the very nature of colour perception itself. For example, Bubl, Kern, Ebert, Bach, and Tebartz van Elst (2010) found that participants with major depression have altered perception of luminance (see below). Major depression is a common mood disorder which affects approximately 10% of the population at any one time (Office for National Statistics, 2000) and whose predominant symptom is persistent low mood (Hammen, 1997) or a lack of joy (Peters, Nicolson, Berkhof, Delespaul, & de Vries, 2003). In this sense, major depression might be considered an extreme extension of sadness, and indeed, both involve overlapping brain regions (in both instances, negative mood state is associated with decreased activity in right prefrontal cortex and increased activity in the subgenual cingulate; Mayburg *et al.*, 1999). Given our interest in mood, it is relevant to consider how depression or other clinical mood states might also relate to different colour qualities. Bubl *et al.* (2010) looked at vision in (non-synaesthete) participants with major depression by assessing their contrast gain. Contrast gain refers to the ability to differentiate objects from their surroundings based on their luminance contrast; an example of high contrast, for example, is the appearance of black letters on white paper. Bubl *et al.* (2010) evaluated the ophthalmologic response of the retina when healthy or depressed participants viewed stimuli of differing contrasts. They found significantly lower retinal contrast gain in depressed patients, suggesting that when people are depressed, they are less able to perceive luminance contrasts in the visual world. The same researchers also showed that people with depression had lower contrast discrimination performance in a behavioural task (Bubl, Tebartz van Elst, Gondan, Ebert, & Greenlee, 2009). Finally, Carruthers, Morris, Tarrier, and Whorwell (2010) found that people in certain clinical mood states are also drawn to different types of colours in a systematic way. They asked healthy participants, anxious participants and participants with depression to select their colour preferences from a colour palette. Both depressed and anxious individuals were more drawn to the achromatic end of the spectrum – particularly to the colour grey. This suggests that low clinical mood states may relate not only to changes in luminance detection but also to lower saturation (i.e., 'chroma') in colour preferences.

The review above has shown that mood states are systematically linked to colour in three different populations: healthy individuals, clinical population (with depression or anxiety) and synaesthetes. Healthy individuals associated negative emotional moods with low saturation and luminance, both as children and adults. Depressed and anxious individuals are drawn to low saturated colours, and the former experience poor contrast sensitivity to luminance. Finally, synaesthetes who are specifically triggered by mood

states as inducers experience lower saturated/ luminant colours for negative valence words.

This study

Above we reviewed the role of mood/emotion in synaesthesia and in colour associations more generally. In this study, we explore whether it can also play a role in variations in synaesthetic colours, even for a type of synaesthesia not generally linked to emotional qualities. Grapheme-colour synaesthesia is considered on the whole to be (1) consistent over time; and (2) devoid of emotional influences (at least in the *triggering* of colour; see also Callejas, Acosta, & Lupiáñez, 2007 and Hochel *et al.*, 2009 for a discussion of how synaesthetes might appraise the un/pleasantness of their synaesthesia, once triggered). Here we ask whether changes in mood state can nonetheless influence grapheme-colour synaesthesia, to bring about subtle changes in synaesthetic concurrent colours over time. In Experiment 1, we examine day-to-day changes in mood in a non-clinicial population of synaesthetes. In addition, since transient sadness and major depression recruit similar brain regions and have similar impact on the association of colour with mood, we will also consider the role of non-clinical depressive traits (Experiment 1) and clinical anxiety disorder (Experiment 2). From our review of colour in clinical/non-clinical mood states above, we predict that positive mood states may be associated with more luminant/ saturated colours, while negative mood states may associate with darker/ less saturated colours. Given these strong predictors for saturation and luminance, we focus on these two features in the current article, to the exclusion of hue (but we are considering the impact on hue in other analyses for future publication).

EXPERIMENT 1

In the study below, we elicited synaesthetic colours for letters from a group of grapheme-colour synaesthetes. Each synaesthete selected their colours from an on-screen colour palette, and did so twice, separated by a period of approximately 3 weeks. Participants were instructed to enter each session only when in a naturalistically different mood: positive on one occasion and negative on another. We confirmed their mood levels using mood questionnaires, and then compared their synaesthetic colours across the two states of mood, in terms of changes in saturation and/or luminance. We used questionnaires to evaluate both non-clinical mood state, and depressive traits. We predicted that the positive mood state would be associated with higher saturation and luminance in synaesthetic colour, while the negative mood state would be linked to lower saturation/luminance levels.

Methodology

Participants

Participants were 24 native English-speaking grapheme-colour synaesthetes who experience coloured letters (mean age: 45.8 years, SD = 13.0; 21 females). Participants were recruited from the Sussex-Edinburgh Database of Synaesthete Participants, and had previously been verified as genuine cases using the 'gold standard' measure of consistency over time (e.g., Baron-Cohen *et al.*, 1987). In this previous verification stage, participants were given synaesthetic triggers as stimuli (in this case, a list of 26 letters) and were

required to verbally report their synaesthetic colours. They were retested without warning after approximately 2 months, and their mean consistency score was 91.7% (*SD* = 12.6). This performance was significantly higher than non-synaesthete controls (*n* = 40; from Simner *et al.*, 2006) who were only 36.2% consistent (*SD* = 13.8; *t* = 16.1, df = 62, $p < .001$). Synaesthete participants were paid £20 for their involvement in our main study, which was given ethical approval by the local ethics board at the University of Edinburgh. Since their genuineness was established as part of other studies, this took place sometime before this study – in some cases, several years earlier – and so would not have influenced performance in the current task (in which synaesthetic colours are also elicited).

Materials

To remove geographical restrictions on testing, our experiment was generated via an online testing platform, created using a survey builder website. The experiment had three components: a test of mood (the Positive and Negative Affect Schedule - Expanded form; PANAS-X; Watson & Clark, 1999), a test of depressive traits (the Beck Depression Inventory – II (BDI-II; Beck, Steer, & Brown, 1996) and a test to elicit synaesthetic colours. These are described in brief below.

PANAS-X

This mood test requires the participant to read 60 emotive words, such as 'distressed' or 'enthusiastic'. Participants were asked to rate how relevant each word was to their current mood state on a 5-point scale, ranging from 'not at all' to 'extremely', with one rating per word. For this study, we focussed on the PANAS-X positive and negative affect scales. Each scale is made up of 10 emotive words (e.g., *enthusiastic* and *distressed*, for positive and negative affect respectively).[1] Stimuli were presented in an order stipulated by PANAS-X, which ensures that words are well distributed and not clustered into emotion subgroups.

BDI-II

The BDI-II (Beck *et al.*, 1996) is designed to determine the presence and level of depressive symptoms. In this test, participants were presented with 21 sets of statements, each set comprising four sentences which are similar but represent a decline in well-being from the first statement to the last. For example:

- I do not feel sad
- I feel sad much of the time
- I am sad all the time
- I am so sad and unhappy that I can't stand it

Synaesthetic letter-colour test

In this sub-test, participants were presented with each letter, one at a time in a random order, and were required to select their synaesthetic colour from an accompanying

[1] *The words within the PANAS-X are also further divided into additional subgroups: four negative (*fear, hostility, guilt, sadness*) three positive (*joviality, self-assurance, attentiveness*) and four neutral (*shyness, fatigue, serenity, surprise*). However, given the small number of words in each sub-group, and our relatively small sample size, we do not consider these subgroupings for this study.*

electronic colour palette. This palette showed colour graduations within a square frame, where hue changes horizontally, and saturation changes vertically. Participants can select a colour by clicking anywhere in the frame. Luminance was manipulated by a separate 'luminance bar' placed vertically to the right of the palette, which could be dragged up and down using the mouse. The selected colour is displayed in a separate display below the palette, and encoded numerically to quantify its hue, saturation and luminance.

Procedure

Participants were informed they would be taking part in an online study which they were to complete by visiting our website twice (i.e., at two separate times). Participants were asked to log onto the website to take part in Session 1, but in particular, only when they felt in a specific mood state. Half the participants were asked to take part in the first session when they felt in a positive mood and the other half when they felt in a negative mood. This is the first time a mood manipulation of this type has been used when testing synaesthetes. The participants were informed that the test would take approximately an hour for each session, and that they were to complete all elements of the test during each session. This was to ensure we had their colour responses for a given session time-linked to their mood/depressive responses.

The first page of the survey informed participants of their ethical rights. Participants then consented with a check box and provided their name, date of birth and date of completing the survey before proceeding to the study. Participants first completed the PANAS-X, then the BDI-II, then the colour palette test. The palette was accessed using a link to an accompanying webpage. Participants selected their colours for letters and transferred the numeric codes into the main survey screen. After this test was completed, the session ended. Participants were contacted 3 days later with the link to the second survey, asking them to be in the alternative mood state when entering it. Therefore, those who had completed the first survey in a positive mood were asked to be a negative mood, and those who had completed the first survey in a negative mood were asked to complete the second survey in a positive mood. The second survey was identical to the first survey.

Although the participants were not explicitly informed that mood was the factor under investigation, they were asked to be in a particular mood state to complete each test. During debriefing, participants reported they found this instruction straight-forward, and we took this approach to profit from natural variances in mood states (rather than to attempt to vary mood states artificially within the test itself – see discussion). On average, participants' two testing sessions were separated by 23.3 days (SD = 14.0).

Results

Coding

Each test was coded prior to data analysis as follows.

PANAS-X

Responses to each word (e.g., *distressed*) are coded 1 to 5, from 'not at all' and 5 to 'extremely'. Our analyses are based on the positive and negative affect scales, each of which contains 10 words. The score for each scale is calculated as the mean score across

the words in each list, giving a maximum possible score of 5.0 (with 1.0 as the minimum). For example, a participant might achieve a total of 32 points by summing each Likert response across the 10 words in the positive affect scale. Their mean final score for this scale would therefore be 3.2.

BDI-II

The BDI-II is scored by first coding each answer according to the level of response: each level is given a score of 0–3, with 0 being the neutral statement and 3 being the most negative statement. The final score is generated by adding these sub-scores scores together to get overall depression score, with a maximum possible score of 63 (with 0 as the minimum).

Synaesthetic colour letter test

The participants' colour choices generated a unique 6-digit alphanumeric code, which represents its hue, saturation and luminance levels. The values for luminance were averaged across all 26 letters, for each subject, and the same process was also used for saturation data.

In our analyses below, we first examine whether we successfully manipulated mood across our two sessions and then examine the influence of mood on synaesthetic colours, first by luminance and then saturation. Prior to all analyses, we first checked the distribution of data using a Kolmogov-Smirnov test, and used parametric and non-parametric analyses as required.

Analyses

Did we successfully manipulate mood?

We compared mean scores for negative affect and then positive affect across our two conditions: *Requested positive mood condition* versus *Requested negative mood condition*. Distribution curves show that negative but not positive affect scores were non-normally distributed and so we use a Wilcoxon signed rank test and a paired sample t-test, respectively. Table 1 shows that, as anticipated, the *Requested negative mood condition* generated higher negative affect scores than the *Requested Positive mood condition* ($Z = -3.8$; $p < .001$), as well as lower *positive* affect scores ($t = 9.4$, df = 23, $p < .001$), and higher BDI-II depression scores ($Z = -4.7$; $p < .001$).

Since scores indicate participants were in the appropriate mood states within each testing condition, we assume henceforth that differences across conditions in synaesthetic colour reflect changes in mood. (We therefore also henceforth refer to our

Table 1. Mean scores across testing conditions in negative/positive affect (from the PANAS-X test) and in depressive traits (from the BDI-II test). There were two testing conditions: participants were instructed to take part when their mood was either low (requested negative mood condition) or high (requested positive mood condition). BDI-II depression scores are 'Minimal' below 14, and 'Mild' at 14–19

	Requested negative mood condition	Requested positive mood condition
Mean negative affect	2.1	1.3
Mean positive affect	2.2	3.3
BDI-II	15.7	5.0

Requested positive/negative mood conditions as simply our *Positive/Negative mood conditions*).

Does mood influence synaesthetic colours?

Below we examine the synaesthetic colour chosen by participants in each mood condition, first by saturation data then by luminance data. The mean saturation of synaesthetic colours was 161.6 in the Positive mood condition (SD = 28.1) and 159.6 in the Negative mood condition (SD = 30.1), showing no significant difference (t = .46, df = 23, p = .7). However, the mean luminance of synaesthetic colours was 131.5 in the Positive mood condition (SD = 21.4) and 127.4 in the Negative mood condition (SD = 20.8; see Figure 1), and this difference was significant (t = 2.4, df = 23, p = .0248; see Figure 1).

What element of mood causes changes in synaesthetic colours?

Having established that changes in synaesthetes' moods affect their synaesthetic colours, we sought to explore what elements of mood were responsible: positive affect, negative affect, or depressive traits. For this we ran a simultaneous entry multiple regression analysis using all three as predictor variables, with luminance as the outcome variable in one model, and saturation in another. Our predictor and outcome variables were each calculated for each subject as the mean across both their testing sessions. We point out that our sample size is small for regression modelling and so our conclusions here are presented as tentative.

Despite our group effect above, there was no significant model relating mood/depression scores as predictors for our luminance data (F = 1.1, df = 2, p = .4). However, for our saturation data, we found a near-significant model (F = 2.6, df = 2, p = .08).

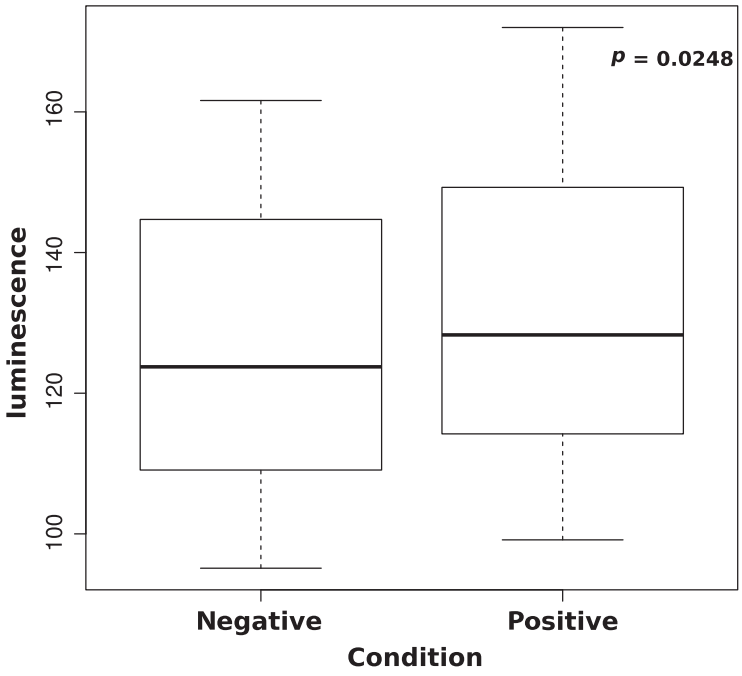

Figure 1. Mean luminance of synaesthetic colours from grapheme-colour synaesthetes in positive versus negative mood. The lower and upper box limits first and third quartiles, and bars delimiting lowest and highest values.

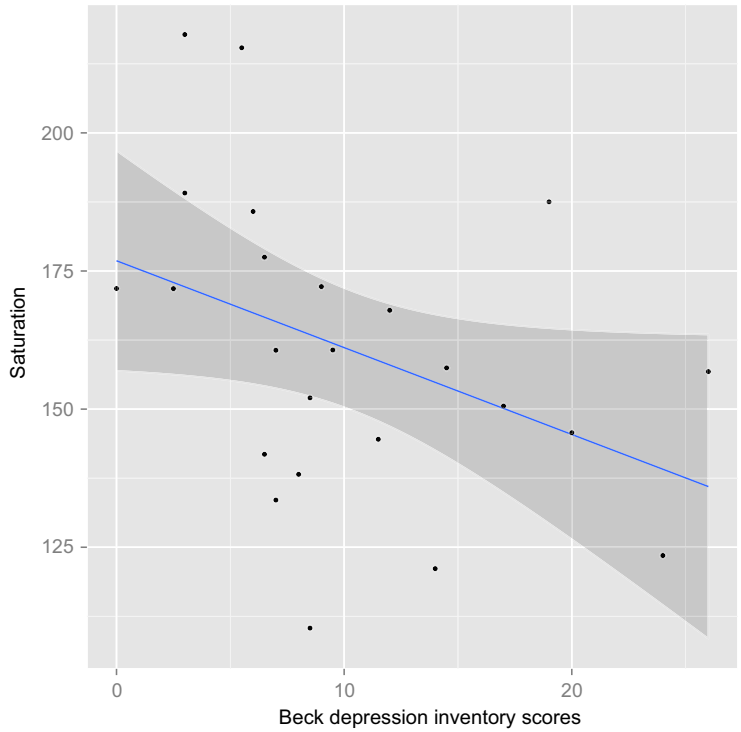

Figure 2. Scatterplot showing a near significant ($p = .08$) inverse correlation between the depressive traits of grapheme-colour synaesthetes, and the saturation of their synaesthetic colours. Depressive traits are scored on the Beck Depression Inventory – II. The graph also shows the best-fit line and 95% confidence interval surrounding it.

Within this model, only one predictor approached significance, and this was depression scores from the BDI-II (ß $= -.60$, $t = -1.8$, $p = .08$) which was negatively related to the saturation of the synaesthetic colour. In other words, as depressive tendencies increase, the synaesthesia's saturation tended to decrease. We illustrate this relationship in the scatterplot shown (see Figure 2) with best-fit line shown.

Discussion

Our study has shown that the synaesthetic colour experiences of grapheme-colour synaesthetes are influenced by their mood. We requested that participants completed our tests in two different mood states, and confirmed those mood states with mood questionnaires. We found that participants reported significantly less luminant synaesthetic colours in the negative mood state compared with the positive mood state. We also found a suggestion that the saturation of synaesthetic colours might be influenced by depressive traits: there was a trend towards lower saturation when depressive traits were higher. In the study below, we extend this finding to a clinical mood disorder, and we also address a methodological concern arising from Experiment 1.

EXPERIMENT 2

There was a methodological concern arising from Experiment 1, which we aim to address in a second study here. Our concern is to verify that we did not induce strategic effects in our first study simply by having asked participants to be in a particular mood. Our method above had advantages because it allowed relatively naturalistic mood differences, but it

also may have alerted our participants to the nature of our investigation. In the study below, therefore, we test the effect of mood without drawing attention to this feature. In Experiment 2, we screened a large random sample of the population to identify the synaesthetes among them, and we also had participants fill out a health questionnaire. Embedded in the questionnaire was an option to indicate whether the participant was suffering from anxiety disorder. Anxiety disorder is a condition in which sufferers experience generalised worry and anxiety, not (necessarily) connected to recent events. Commonly regarded as belonging to a spectrum of mood related disorders, symptoms include feelings of threat, irritability and tension (Tyrer & Baldwin, 2006). In this study we will investigate whether synaesthetic colours are systematically different (e.g., less luminant) for those with (vs. without) anxiety disorder.

Methodology

Participants

Our participants were 34 English-speaking grapheme-colour synaesthetes (mean age = 21.9, *SD* = 5.8, 20 females), six of whom had (self-reported) anxiety disorder (mean age: 20.8, SD: 2.8; 3 females) and the remaining 28 reported did not (mean age: 22.1 years, *SD* = 6.3; 17 females). Participants were recruited via a large-scale screening of 2847 members of the general population (mean age: 28.4, *SD* = 14.3; 1530 female) for grapheme-colour synaesthesia (see Carmichael, Down, Shillcock, Eagleman, & Simner, 2014 for further information). Our subjects were verified as synaesthetes using the objective test described below.

Materials and procedure

Our procedure allowed us to identify a group of randomly sampled synaesthetes, tested objectively, and also to verify which of those synaesthetes had anxiety disorder and which did not. Our procedure also allowed us to determine our synaesthetes' grapheme-colours, so that we could then compare these across individuals with and without anxiety disorder.

All 2847 participants (including the 34 synaesthetes that would ultimately be found among them) were sent to an online testing website where they completed a 2-stage test. The first stage was a health questionnaire in which participants indicated (inter alia) whether they did, or did not suffer from anxiety disorder. This health questionnaire listed 24 conditions in total (e.g., anxiety disorder, asthma, migraine) meaning that our focus of interest was sufficiently hidden.

The second stage of testing was a short questionnaire which described grapheme-colour synaesthesia and asked whether participants thought they might experience this. For those who responded in the affirmative, an objective test followed. As in Experiment 1, this objective test for synaesthesia was based on consistency-over-time as the behavioural hallmark of synaesthesia. Also as stated earlier, synaesthetes were identified as those who were significantly more consistent than non-synaesthete controls. Our consistency test was this time performed via the online diagnostic site synesthete.org (see Eagleman, Kagan, Nelson, Sagaram, & Sarma, 2007 for methods, also Carmichael *et al.*, 2014). This site not only verifies synaesthesia, but also gathers synaesthetic colours in a way that can later be analysed according to saturation and luminance values. In this battery, graphemes were presented three times each in a randomised order and participants were required to select their synaesthetic colour for each grapheme from a palette of 256 × 256 × 256 colours (see Eagleman *et al.*, 2007 for details of this

interface). In this battery, the mean colour distance across the three presentations of each grapheme is converted to a standardized score, where a score less than 1 represents a distance small enough to indicate synaesthetic status (see Eagleman *et al.*, 2007 for details). All 34 synaesthetes achieved this required score of less than one.

Results

In our analyses we compared the grapheme colours of synaesthetes with and without anxiety disorder. The output of the online testing site was the colours of graphemes encoded as RGB (red, green, blue) vector values. We first converted these values to their corresponding hue, saturation and luminance. We then averaged the luminance of all graphemes for each subject across all their responses (maximally, this was a mean across all three presentations of each letter a–z and each digit 0–9, although participants were free to omit graphemes that had no synaesthetic colour). We then repeated this for the saturation values, and finally compared both these types of means across participant groups. The saturation of synaesthetic colours was not significantly different for synaesthetes with anxiety disorder ($M = 209.4$, $SD = 12.6$) compared to those without ($M = 191.0$, $SD = 35.9$; $t = 1.2$, df = 32, $p = .2$). However, synaesthetes with anxiety disorder had significantly darker (i.e, less luminant) colours ($M = 102.3$, $SD = 21.5$) than those without anxiety disorder ($M = 121.9$, $SD = 20.4$; $t = -2.1$, df = 32, $p = .04$). This effect is illustrated in Figure 3.

Discussion

Our study has shown that the synaesthetic colour experiences of grapheme-colour synaesthetes are different for those with and without anxiety disorder. Those

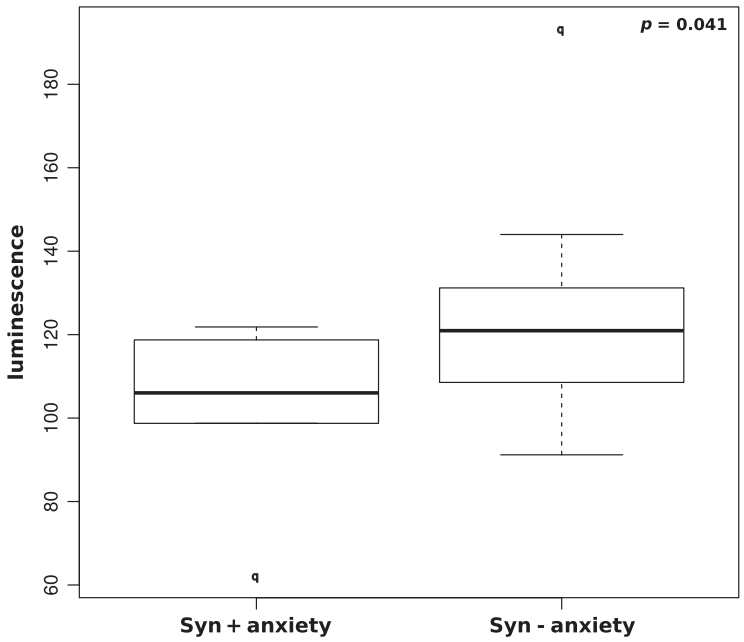

Figure 3. Mean luminance of synaesthetic colours from grapheme-colour synaesthetes with and without anxiety disorder (shown as *Syn + anxiety* and *Syn − anxiety*, respectively). The lower and upper box limits first and third quartiles, and bars delimiting lowest and highest values. Outliers are shown as cases marked 'q'.

self-reporting this condition had significantly darker synaesthetic colours (i.e., lower in luminance). We discuss the implications of our findings across both studies below.

GENERAL DISCUSSION

In our studies, we elicited synaesthetic colours for letters from groups of grapheme-colour synaesthetes, using an on-screen colour palette and converting colour selections into numeric values for hue, saturation, and luminance. In Experiment 1, synaesthetes selected their colours twice, separated by a period of several weeks, and were instructed to enter each session in a naturalistically different mood: positive on one occasion and negative on another. In each session we confirmed their mood state using the PANAS-X (Watson & Clark, 1999) and the BDI-II (Beck *et al.*, 1996) which quantify both non-clinical mood states and also depressive traits. Our questionnaire data confirmed that participants were in a significantly more positive mood when this had been requested of them. We then compared our participants' synaesthetic colours across the two (positive vs. negative) mood states in terms of changes in saturation and luminance. We found that synaesthetic colours in the negative mood state were on average significantly less luminant than in the positive mood state. A second, more speculative multiple regression analysis looked at which features of mood might influence colour changes: positive affect, negative affect (PANAS-X subscales thereof), and depressive traits (from the BDI-II scale). This analysis suggested that saturation might also be influenced by depressive traits: there was a trend towards lower saturation when depressive traits were higher. However, given that this latter trend failed to reach significance at the conventional alpha level, we will not consider it further here.

In Experiment 2 we extended this finding with another group of grapheme-colour synaesthetes. We showed that mood can alter synaesthetic colours not only when it fluctuates in non-clinical everyday life (Experiment 1), but also when it alters more dramatically, in anxiety disorder (Experiment 2). We showed that synaesthetes with self-reported anxiety disorder experience significantly darker synaesthetic colours compared to synaesthetes not suffering from anxiety disorder. Second, we addressed a methodological limitation from Experiment 1, where our request for participants to take part in a given mood state may have alerted them to the aims of our study. In Experiment 2, the aims of our study were sufficiently hidden by asking about mood (i.e., anxiety disorder) among 23 other medical conditions. In evaluating our findings, we first point out that the colour changes that arose with mood/anxiety were not large: hence our synaesthetes were still highly consistent over time, and significantly more consistent than controls. Similarly in Experiment 1, colours for letters were overall still very similar across the two testing sessions: their mean luminance changed by only 2 percentage points (i.e., 4.0/255) and their mean saturation by only 0.8 percentage points (i.e., 2.0/255). Hence although synaesthetic colours are influenced by mood states, they shift only marginally when mood varies within a normal (i.e., non-clinical range). Nonetheless, these differences were larger in Experiment 2, where the mood manipulation was more extreme: the colours of synaesthetes reporting anxiety disorder were more than 7 percentage points (19.6/255) darker in luminance. In both experiments, these differences were systematic and statistically significant, but the more extreme mood change caused the more extreme difference in synaesthetic colour.

We point out that our findings fit with prior literature relating mood to colour in both the general non-synaesthetic population, and in clinical non-synaesthetic populations. We

saw above that healthy adults and children associate negative emotional moods with low saturation and luminance (Boyatzis & Varghese, 1994; Hemphill, 1996; Manav, 2007) and that depressed and anxious individuals are drawn to low saturated colours (Carruthers *et al.*, 2010). Our own findings reflect the same direction of effects in *synaesthetic* colours. Our findings also fit with one previous study showing that an emotionally mediated synaesthete experienced darker and less saturated colours from negatively valent words (Ward, 2004). In all cases therefore, negative mood states were linked with low luminance and/or saturation of colours. If we assume that our findings reflect similar mood changes in grapheme-colour synaesthesia, what might be at the root of these changes? One possibility is that mood may be influencing synaesthetic colours via arousal. Another possibility is that changes in synaesthetic colour by mood are mediated by attention. It is known that attention broadens in positive mood states (Compton, 2003; Wadlinger & Isaacowitz, 2006) so may indirectly influence synaesthesia simply because attention on the inducer is in some way heightened. Rich and Mattingley (2013) provide a comprehensive review of how attention influences synaesthetic colours, and one intriguing fact from our own data may speak to this issue: the PANAS has a subscale of attention (comprising the items *Alert*; *Attentive*; *Concentrating*; *Determined*). A post-hoc correlation between luminance and attention reveals a near significant r value ($r = -0.22$, $p = 0.06$). This seems to suggest that one aspect of mood linked to changes in synaesthetic colour may indeed be attentiveness.[2] Nonetheless, this result is just a trend based only on four items, and so we leave it to future studies to explore the link between attention and synaesthetic colour variation in more detail.

We might also consider our findings in terms of the neurological bases for mood, and also for synaesthesia. Mood and emotional processing has been localised to a number of regions, most particularly the limbic system, including the hippocampus, septal nuclei, amygdala, cingulate gyrus, mammillary bodies and hypothalamus (for review see Martin, 2006). The amygdala has been particularly implicated in processing the emotional importance of stimuli, for example, in behavioural and cognitive response to stimuli that induce fear (Adolphs, Tranel, Damasio, & Damasio, 1995; Davis, 1992). However, the amygdala is also believed to be important for perception and attention (Anderson & Phelps, 2001; Williams, McGlone, Abbott, & Mattingley, 2005). For example, Young, Scannell, Burns, and Blakemore (1994) found that the amygdala has connections to numerous cortical regions, including sensory regions. It is possible therefore that differences in emotional processing induced by mood changes have direct influences on visual, and other perceptual functions. Alternatively, we might consider instead one area in particular in visual cortex, V4, which has been directly implicated in synaesthetic colours (e.g., Hubbard, Arman, Ramachandran, & Boynton, 2005), but has also been linked to the feature of contrast gain (Gardner *et al.*, 2005). We saw above that contrast gain is altered in depressive mood states (Bubl *et al.*, 2009, 2010). It may be possible therefore, that the same mood-linked processes that alter contrast gain may have simultaneous effects on synaesthetic colour-selective regions in the brains of synaesthetes.

Following the completion of our own study, a related article has very recently appeared in press which is strongly compatible with our own (Dael, Sierro, & Mohr, 2014). These authors, like us, have questioned the role of mood, emotion and affect in

[2] *We did not conduct further analyses of PANAS subscales because we had no other a priori assumptions, and therefore chose to avoid the corrections required by multiple comparisons, given also our small sample size.*

synaesthesia. Their article is an interesting opinion piece hypothesising that emotion might hold sway in synaesthesia not only as an inducer or concurrent, but also as 'neither the inducer nor the direct concurrent, but a moderator or mediator of the synesthetic coupling' (Dael *et al.*, 2014). We suggest that our own study now offers precisely the empirical data to support this speculation. Our results show empirically that mood can indeed mediate the synaesthetic experience, and we point the reader to Dael *et al.* (2014) for an excellent and comprehensive review of mood and emotion in both synaesthetic and non-synaesthetic perception.

In evaluating our study we would like to present two possible alternative explanations of our findings, and evaluate these alternatives in the light of the data at hand. One key finding to consider when interpreting our study is that prior research has shown mood changes can influence perception itself (e.g., Bubl *et al.*, 2009, 2010). Given this, an alternative interpretation of our data might be that people with synaesthesia in fact have rigidly consistent synaesthetic colours, but that mood changes alter their ability to *indicate those colours via our task*. In other words, it may be that their interface with our colour wheel was compromised by changes in *real-world* perception, but their synaesthesia itself remained unchanged. A closer inspection of the direction of our data appears to speak against this possibility, and we explain this below.

Bubl *et al.* (2009, 2010) found that individuals with depression had lower contrast sensitivity, and although it is difficult to interpret this directly in terms of *absolute* luminance perception, it may be that this represents a shift towards under-perceiving lightness (i.e., perceiving lightness as being less light). This would certainly follow from decision studies linking negative mood states with lower luminance (e.g., Boyatzis & Varghese, 1994; Hemphill, 1996; Manav, 2007; see above). Furthermore, depressed individuals do indeed explicitly report that light appears dimmer (Friberg & Borrero, 2000), and perceptual judgements of brightness are lessened following negative (vs. positive) affect judgements (Meier, Robinson, Crawford, & Ahlvers, 2007). If we extend this conclusion to this study, we might then wish to ask whether our findings could simply be explained as our participants perceiving our screen colours as less bright when in a bad mood. If so, our findings would be unrelated to changes in synaesthesia at all. However, the direction of our findings argues against this, because we in fact found the opposite effect: selected colours were lower in luminance, but should have been higher if the screen simply appeared darker in a perceptual sense. In other words, if perception rendered the screen perceptually darker, subjects should have selected higher (not lower) levels of luminance in order to compensate. They did not: luminance fell in the negative mood state suggesting this represented an accurate selection of an altered internal colour, rather than an impaired real-world selection of an unchanged internal colour.

A second alternative interpretation of our findings is that our mood caused changes not in synaesthetic colour, but in participants' positioning of the curser on our electronic palette. An anonymous reviewer has pointed out that luminance in both studies was manipulated vertically with lightness at the top. This leaves open the possibility that poor mood state did not affect luminance, but that instead it affected the height selected on the screen (via English spatial metaphors related to mood such as: 'I feel down today'). We investigated this possibility and found it was not the case. From the same $n = 2847$ sample of Experiment 2, we examined the data of all subjects who attempted our test for synaesthesia but were ultimately not synaesthetes. Remembering that synaesthetes are those with a standardized score in our objective consistency test of <1 (see Methods), we now selected as a group of non-synaesthete controls all those who scored >2. By coincidence, this group had the same make-up as our synaesthetes ($n = 34$, of whom six

had anxiety disorder). These controls completed the identical task as synaesthetes, under exactly the same testing conditions, with exactly the same instructions, and all were recruited by exactly the same method. If anxiety/ negative mood state causes people to place the cursor lower on our electronic palette we would expect again to find that those with anxiety disorder selected colours with a lower luminance. They do not: people both with and without anxiety disorder placed the cursor at the same height (i.e., they choose the same luminance) as each other. The mean luminance for those with and without anxiety disorder was 110.2 [SD = 24.0] and 107.3 [SD = 12.8] respectively (t = 0.27, df = 5.51, p = 0.8). Hence only for those with synaesthesia, does anxiety disorder lead to lower luminant (synaesthetic) colours.

In conclusion, we have found that mood states influence concurrent colours in grapheme-colour synaesthesia, and that negative states in particular give rise to colours that are lower in luminance, either as part of everyday non-clinical fluctuations in mood (Experiment 1), or as part of more extreme fluctuations of the type found in anxiety disorder (Experiment 2). We also found a trend to suggest that higher depressive traits within the non-clinical range may give rise to lower saturated synaesthetic colours. These results have implications for research into synaesthesia not only because they provide novel information about how mood alters internal perceptions, but because they challenge the traditionally held view that adult synaesthetic colours are consistent over time. We invite future researchers to explore further when and how synaesthetic colours might vary over time, as a key to better understanding the limits of this unusual condition.

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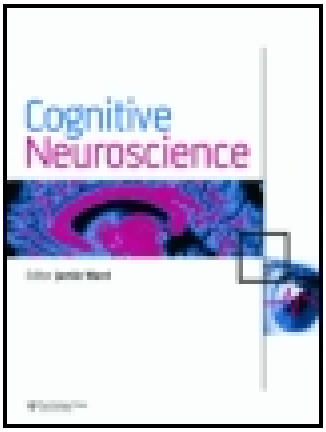
Received 11 December 2013; revised version received 24 July 2014

Appendix I

Is synaesthesia a dominantly female trait?



Cognitive Neuroscience
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/pcns20

Is synaesthesia a dominantly female trait?

Julia Simner[ab] & Duncan A. Carmichael[bcd]
[a] School of Psychology, Pevensey Building, University of Sussex, Falmer, UK
[b] Department of Psychology, University of Edinburgh, Edinburgh, UK
[c] Institute for Adaptive & Neural Computation, University of Edinburgh, Edinburgh, UK
[d] Division of Psychiatry, University of Edinburgh, Royal Edinburgh Hospital, Edinburgh, UK
Accepted author version posted online: 03 Mar 2015.Published online: 09 Mar 2015.

To cite this article: Julia Simner & Duncan A. Carmichael (2015): Is synaesthesia a dominantly female trait?, Cognitive Neuroscience, DOI: 10.1080/17588928.2015.1019441

To link to this article: http://dx.doi.org/10.1080/17588928.2015.1019441

COGNITIVE NEUROSCIENCE, 2015, http://dx.doi.org/10.1080/17588928.2015.1019441

Is synaesthesia a dominantly female trait?

Julia Simner[1,2] and Duncan A. Carmichael[2,3,4]

[1]School of Psychology, Pevensey Building, University of Sussex, Falmer, UK
[2]Department of Psychology, University of Edinburgh, Edinburgh, UK
[3]Institute for Adaptive & Neural Computation, University of Edinburgh, Edinburgh, UK
[4]Division of Psychiatry, University of Edinburgh, Royal Edinburgh Hospital, Edinburgh, UK

Synaesthesia is a familial condition that gives rise to unusual secondary percepts. We present a large-scale prevalence study which informs our ideas on whether the condition is more prevalent in men or women. A number of studies over the last 20 years have suggested the condition is found more commonly in women, with up to six times more female synaesthetes than male. Other studies attributed this female bias to merely a recruitment confound: women synaesthetes may be more likely to self-refer for study. We offer two pieces of evidence that there is no extreme female bias in synaesthesia: first we re-analyse previous reports of very large female biases to show again that they likely arose from self-referral or other methodological issues. Second, we present the largest published prevalence study to date on *grapheme→colour synaesthesia* in which our prevalence (1.39% of the population) replicates our earlier estimates (and in which we demonstrate no strong female bias even with sufficient power to detect such a difference.

Keywords: Synaesthesia; Prevalence; Sex ratio; Synesthesia.

For people with synaesthesia, stimuli are experienced with unusual secondary associations (e.g., hearing sound triggers colours in the visual field; Ward, Huckstep & Tsakanikos, 2006). Synaesthesia is a multi-variant condition with an estimated 65 (Day, 2005) to 150 (Cytowic & Eagleman, 2009) known sub-types, depending on which modalities are linked (e.g., sound triggering colours, taste triggering touch etc.). One key question is how common synaesthesia is, and whether it affects men and women differently. Early estimates described the condition as extremely rare (e.g., 1 in 250,000) and very strongly female dominant (with a 6:1 ratio; Baron-Cohen, Burt, Smith-Laittan, Harrison & Bolton, 1996). Later studies have called into question both these claims and we examine these issues in the current paper.

Despite a relatively contentious history, the question of synaesthesia's prevalence appears to now be reasonably well understood. Early estimates of prevalence varied widely at least partly because researchers were focussing on different sub-types or using different definitional criteria (Ramachandran & Hubbard, 2001). However, even in studies that aimed to report the prevalence of all forms of synaesthesia, estimates ranged from 1 in 4 (Calkins, 1895; Domino, 1989; Uhlich, 1957), to 1 in 10 (Rose, 1909), 1 in 20

Correspondence should be addressed to: J. Simner, School of Psychology, Pevensey Building, University of Sussex, BN1 9QJ, UK. E-mail: j.simner@sussex.ac.uk

Julia Simner and Duncan A. Carmichael contributed equally to this work

DAC was supported in part by grants EP/F500385/1 and BB/F529254/1 for the University of Edinburgh, School of Informatics Doctoral Training Centre in Neuroinformatics and Computational Neuroscience (www.anc.ac.uk/dtc) from the UK Engineering and Physical Sciences Research Council (EPSRC), UK Biotechnology and Biological Sciences Research Council (BBSRC), and the UK Medical Research Council (MRC).

The research leading to these results has received funding for author JS from the European Research Council under the European Union's Seventh Framework Programme (FP/2007-2013) / ERC Grant Agreement n. [617678].

(Galton, 1883), 1 in 200 (Ramachandran & Hubbard, 2001), 1 in 2000 (Baron-Cohen et al., 1996), and 1 in 25,000–100,000 (Cytowic, 1993, 1997). One problem was that many of these early estimates were essentially 'best guesses'. Nonetheless, these early studies served the important purpose of stimulating research on synaesthesia's prevalence and inspired the first empirical assessments which followed thereafter.

The first prevalence study of its kind in the modern literature (Baron-Cohen et al., 1996) assessed the occurrence of synaesthesia by placing adverts in two local newspapers in Cambridge, UK, calling for synaesthetes to come forward. The advert described several types of synaesthesia (sound [including linguistic sounds]→colour, touch/taste/smell→vision/sound) and identified two types of synaesthesia in respondents, now known commonly as *grapheme-colour synaesthesia* (experiencing colours from letters and/or digits) and *music-colour synaesthesia* (experiencing colours from sounds such as music). By comparing the number of synaesthetes who came forward (and who were subsequently verified as genuine using an objective test; see below) against the circulation figures of the newspapers, Baron-Cohen and colleagues concluded that synaesthesia was at least as common as 1 in 2000 people (i.e., 0.05%). However, their methods would have greatly underestimated the true prevalence because they relied on synaesthetes making the effort to come forward in response to a newspaper advert. For this reason the authors of that study were careful to point out that their figure was only a lower estimate, although their data has almost always been misrepresented in the following literature as an absolute estimate. A small number of studies in the historical literature had avoided the problems of self-referral by individually questioning every member of a participant pool, although they established prevalence only subjectively (at 6.7–23.0%: Calkins, 1895; Domino, 1989; Rose, 1909; Uhlich, 1957) by relying on self-declaration only, which is an approach known to over-estimate prevalence (Simner et al., 2006). Hence, one set of studies tends towards a conservative estimate and the others towards an overly-liberal one.

Our own study in 2006 addressed these limitations by individually assessing a large number of people (n = 1690[1]) and verifying their self-declarations with an objective test of genuineness (see below). These improvements in methodology showed the condition to be far more common than previously thought, affecting 1 in 23 members of the general population across the relatively wide range of synaesthesias tested (Simner et al., 2006). The important element in this study was that synaesthetes were not required to make the effort to self-refer in response to an advert. Instead, a large sample of the general population was individually assessed to find the synaesthetes from among them, and this gave a prevalence of 4.4% of synaesthetes within the general population, for the variants tested. Within this figure, one particularly common form was grapheme-colour synaesthesia, in which colours are triggered by letters and/or digits (e.g., *A* might trigger the experience of red, *B* yellow, and so on). The prevalence of grapheme-colour synaesthesia was 2% (counting those with coloured letters *and/or* digits; or 1.1–1.4% for those with both coloured letters *and* digits). Since the time of this study, these estimates for the prevalence of different forms of synaesthesia have been widely accepted (e.g., Banissy et al., 2012; Bor, Rothen, Schwartzman, Clayton & Seth, 2014; Cohen Kadosh & Henik, 2007; Ward, 2013; Weiss & Fink, 2009).

In contrast to prevalence estimates, the sex ratio of female to male synaesthetes has caused perhaps greater controversy. Several early studies proposed that there was a very strong female bias in synaesthesia, suggesting a possible X-linked dominant mode of inheritance (e.g., Baron-Cohen et al., 1996; Smilek et al., 2002). Indeed, the extent of this female bias in some studies (e.g. 6:1; Baron-Cohen et al., 1996) led researchers to believe that the trait may even be associated with male lethality *in utero* (Bailey & Johnson, 1997; Baron-Cohen et al., 1996). This would in turn suggest that synaesthetes' families should contain more women than men. However, both these claims were subsequently challenged by later studies, and we describe this development below.

Until 2006, the most commonly cited synaesthesia study on prevalence and sex-ratios (Baron-Cohen et al., 1996) proposed a female: male ratio of 5.5:1, and this was followed by a second study (Rich, Bradshaw & Mattingley, 2005) proposing a female bias of 6.2:1. However, both studies based their estimates on the number of synaesthete who self-referred in response to media advertisements (e.g., newspaper adverts). Not only will this method underestimate the total number of synaesthetes in a population (see above) but it is also likely to over-estimate the females. This is because females are known to be more likely than males to come forward to report atypical experiences, and this is

[1]Specifically, 1190 individuals were assessed for grapheme-colour synaesthesia, and a further 500 individuals were tested for 162 different synaesthesias, one also being grapheme-colour synaesthesia. Since the estimates of prevalence for grapheme-colour synaesthesia were approximately equivalent across both populations, Simner et al. (2006) collapsed both population sizes to give a grand total of 1690 people tested.

seen across a range of domains (e.g., Dindia & Allen, 1992). Simner et al. (2006) therefore suggested that a self-referral confound may be responsible for the previously high rates of female synaesthetes in prevalence studies. Indeed, when this potential confound was directly avoided by Simner et al. (2006), we found that earlier studies had indeed apparently over-inflated the proportion of females. As noted above, Simner et al. (2006) specifically did *not* rely on self-referred recruitment in their prevalence estimate, but instead, they individually assessed every member of a large participant pool and used an objective test to identify the synaesthetes from among them. Using this improved methodology we found that that there was no large (e.g., 6:1) bias towards female synaesthetes. Instead, we found a female: male ratio of 1.1:1 when considering a wide range of synaesthesias in a population of n = 500, and a female: male ratio of 0.9:1 when considering grapheme-colour synaesthesia[2] in a population of 1190. Neither of these comparisons showed any significant sex bias.

On the basis of the above literature we might conclude that synaesthesia affects around 1 in 23 individuals and has no very strong sex bias. However, there have been three subsequent challenges to our position. First, a small number of studies continue to cite the prevalence and/or sex ratio from Baron-Cohen et al. (1996) despite the self-referral confound, and this has propagated in the literature a low value of prevalence and a high estimate of female synaesthetes. Second, one subsequent study (Barnett, Newell, Finucane, Asher, Corvin, Mitchell, 2008) has pointed out that the sex differences identified in self-referral more generally (Dindia & Allen, 1992) only account for a slight variation (10%) in men and women's responding, making it possible that very high early estimates for female synaesthetes were at least pointing in the right direction. Third, that same study (Barnett et al., 2008) presented data that were ostensibly free from the self-referral confound, but which continued to show a strong (6:1) ratio of female to male synaesthetes. For these three reasons we return to the issue of sex differences in synaesthesia in the current paper. The position we take is to re-affirm that there is no strong 6:1 ratio of female to male synaesthetes when all self-referral confounds are removed. We do this below by presenting our own very large-scale study free of self-referral, but before then, we also re-evaluate the findings by Barnett et al. (2008). Their findings had been reported as evidence of a strong (6:1) ratio of female to male synaesthetes in data that were presented as being apparently free from the self-referral confounds. We re-evaluate this claim below.

Barnett and colleagues conducted a synaesthesia study of the mode of inheritance, and prevalence of synaesthesic sub-types within families. In their study they looked not only at self-referred probands (i.e., 53 synaesthetes who self-referred to the university in response to a media advert) but also a subset of their family members who were questioned by the proband and/or directly contacted by the researchers. Since family members were tested as well as self-referred probands, Barnett et al. claim their findings are free of a self-referral confound, and they report that "our total sample of 92 confirmed and unconfirmed synaesthetes includes 78 females and 14 males, yielding a female to male ratio of 6:1 in the Irish population" (pg. 877). Below we present several responses to these claims.

First, in their calculations of the female: male ratio, Barnett et al. appear to directly compare their 78 female synaesthetes against their 14 male synaesthetes, concluding that a female: male ratio in synaesthesia of 6:1 exists in the general population (more precisely this would be: $^{78}/_{14}$: $^{14}/_{14}$ = 5.57:1). However, Barnett et al. evaluated twice as many females than males (118 vs. 61) if we include all 179 participants whose status was somehow appraised during their study (i.e., excluding all those with an unknown status). This factor would considerably reduce the absolute proportion of female synaesthetes to males in their estimate for the general population.

A second consideration comes in the claim that Barnett et al.'s findings were not contaminated by a self-referral bias, because they looked not only at self-referred probands but also their families. However, according to our reading of their report, Barnett and colleagues were able to objectively verify the synaesthesia of all their self-referred probands, but only a small portion of their non-proband synaesthetes. Indeed, 81 of their 92 synesthetes overall were either objectively unconfirmed cases (n = 28), or they self-referred in response to an advert (n = 53) and were therefore likely to be *a priori* female-skewed. Equally, when Barnett et al. state there was "no difference… between the sex ratio for probands (46 females and 7 males) and … relatives who did not contact us directly (30 females and 5 males)", we point out that almost 70% of these synaesthete relatives appear to have received no objective test for synaesthesia. As such, almost every member of their cohort were either

[2]This particular reported figure related to grapheme-colour synaesthetes who experience both coloured letters *and* digits (rather than coloured letters and/or digits).

self-referred, or were not verified as synaesthetes by the usual objective standard.

Finally, Barnett et al. (2008) report that 17 families were fully explored as far as all first-degree relatives of the proband and this still gave a "6:1" (pg 885) ratio of female to male synaesthetes (i.e., 45 female synaesthetes and 8 male synaesthetes found within these 17 families). We point out, as above, that 45 female vs. 8 male synaesthetes cannot be interpreted as 6:1 prevalence in the general population without first knowing the sex of each family member tested in those 17 families as a whole which was not provided. We also point out that one third of the synaesthetes discovered within those 17 families (i.e., 17 of the 53 synaesthetes discovered) would have been contaminated by a self-referral confound that strongly skews towards females, because these families centred around 17 synaesthete probands, who self-referred for study. Importantly, 87% of all (n = 53) probands were female, meaning that approximately 87% of the 17 probands in the target families would be females, from what we know is a skewed sampling method. In summary, target families were not selected in a way to be free of an *a priori* recruitment confound because all contained a proband recruited by self-referral (see also Ward & Simner, 2005 for a similar problem). Finally, we point out, as above, that approximately half of the 53 synaesthetes within the 17 target families did not receive an objective test (of consistency) for synaesthesia.

In summary, we conclude that the 6:1 ratio towards female synaesthetes found by Barnett et al. (2008) did not take into account the total number of females categorised overall, or *a priori* confounds in the recruitment of self-referred synaesthetes, and it did not categorise synaesthetes with objective testing throughout. For these reason we conclude that their 6:1 bias towards female synaesthetes was affected, at least to some degree, by self-referral methodology or *other* issues. (Nonetheless, we point out that the study by Barnett and colleagues provided much robust data on *a number of other* epidemiological and cognitive factors within synaesthesia—e.g., transmission of different variants within families, trends in synaesthetic colours. etc.—and it therefore represents a valuable step towards understanding how synaesthesia might manifest itself, beyond this sex issue.) Below we test whether there is a 6:1 female bias empirically when self-referral is removed, but we first conduct a power analysis to confirm the numbers that would need to be tested in order to determine whether such a difference were statistically significant. This is important because previous epidemiological studies of synaesthesia aiming to remove the self-referral confound (e.g., Simner, Harrold, Creed, Monro & Foulkes, 2009; Simner et al., 2006) have tested too few people to provide sufficient power for a statistical comparison of the sexes.

POWER ANALYSIS

The female bias in synaesthesia estimated by Baron-Cohen et al. (1996) was 5.5:1, and by Rich et al. (2005) it was 6.2:1, and by Barnett et al. (2008) it was 5.6:1. These values, repeatedly circling around a 6:1 ratio of female to male synaesthetes, can be tested empirically if there is sufficient power in the number of individuals tested. In order to calculate this we first need to estimate what the individual prevalences of synaesthesia would be for males versus females, given a hypothesised 6:1 difference.

The most robust and widely cited synaesthesia prevalence study to date (Simner et al., 2006), report an overall prevalence of synaesthesia of 4.4% of the population, when testing for 162 different variants. However, there are considerable challenges to identifying so many different types of sub-variants within a single study (see Simner et al., 2006 for discussion) so we instead chose to test for just one variant of synaesthesia in the current study. We chose grapheme-colour synaesthesia since this variant is very well understood, relatively prevalent, and can be tested for using a single standardised computerised method (see below). Below we therefore conduct a power analysis to reveal the number of individuals required for screening in order to identify any 6:1 bias of female synaesthetes with grapheme-colour synaesthesia.

Simner et al. (2006) report the prevalence of grapheme-colour synaesthesia to be 2% (where "grapheme-colour synaesthetes" are those with either coloured letters, coloured digits, or with both). With an assumed sex ratio of 6 female synaesthetes to every male synaesthete, we would expect to find 1.71 female synaesthetes and 0.29 male synaesthetes if we tested 100 members of the population. If we carry out a sample size calculation for a chi-squared test, with standard levels of power at 0.80 and alpha at 0.05, in order to detect a difference in proportion of this magnitude (1.71% versus 0.29%, or proportions of 0.0171 and 0.0029 respectively) a sample of 1810 participants is required for screening (905 females and 905 males). In our empirical study below, we meet—and indeed exceed—this sample size.

EMPIRICAL ASSESSMENT

We individually assessed a very large number of individuals from the general population for

grapheme-colour synaesthesia, avoiding a self-referral bias. Every person was assessed using the behavioural "gold-standard" test which identifies synaesthetes by detecting the most widely accepted core characteristic of synaesthesia. This characteristic is the consistency in the reporting of synaesthetic sensations over time. In grapheme-colour synaesthesia for example, a given letter tends to elicit a consistent synaesthetic colour for any given synaesthete in repeated testing (e.g., *A* might be consistently red, *B* consistently blue, etc.). This consistency-over-time is taken as the behavioural hallmark of synaesthesia in standard diagnostic tests for synaesthesia (see Johnson, Allison & Baron-Cohen, 2013 for review). The mostly widely used version of this test for grapheme-colour synaesthesia is available at an online interface known as the *Synesthesia Battery* (Eagleman, Kagan, Nelson, Sagaram & Sarma, 2007). In this test, participants are required to repeatedly report their synaesthetic associations for the letters A-Z and/or the digits 0–9, each shown three times in a random order. In order for people to be diagnosed as synaesthetes, they must achieve high enough consistency in their colour-choices to show they are significantly better than non-synaesthete controls, who previously performed the same test to provide a robust base-line. This task was used in our own study, and more details are given in Eagleman et al. (2007) and in our methods below.

Participants

We individually screened 3893 participants for grapheme-colour synaesthesia using *The Synesthesia Battery* (2135 female; 1758 male). Their mean age was 28.3 years (SD = 14.2). A further 65 participants were excluded from study because they had entered an obviously false date of birth (e.g., 2013; n = 48) or because they reported too few coloured graphemes for their synaesthesia to be meaningfully evaluated (n = 17; see Eagleman et al., 2007). Participants were unpaid, and our study was approved by the local university ethics board.

Participants were recruited as part of a large-scale, centrally co-ordinated undergraduate research project, described in detail in Carmichael, Down, Shillcock, Eagleman and Simner (2015). In this, every student registered on the 2nd year of the Psychology undergraduate course at the University of Edinburgh between September 2012 and May 2015 acted as a research assistant (RA), each recruiting approximately 8 participants (4 male and 4 female) over 16 years of age. In recruiting participants, we took a number of steps to ensure as random a sample as possible: RAs were required to pre-select their sample, and then approach participants in a targeted way, rather than sending out an advert for self-referrals. Indeed, RAs were required to refrained from recruiting participants via any advert or open calls at all. For example, they could not post the testing URL on social media websites or internet forums. Furthermore, RAs were instructed not to deliberately seek out, nor to avoid, people they knew to be synesthetes and were also instructed not to *a priori* inform participants that the study investigated synesthesia. Instead, they pre-selected their samples to create a pre-determined, non-referred testing cohort, and then individually tested every member of that cohort.

Methods

To screen for grapheme-colour synaesthesia, we used the consistency test from the *Synesthesia Battery* on-line interface (Eagleman et al., 2007), which we cloned with permission from the authors (see Carmichael et al., 2015 for details). Participants were provided with the URL of our online interface and completed the test in their own time.

Our replication of the *Synesthesia Battery* first obtains consent for testing and then records demographic information about participants including age and sex. Participants are then asked whether they experience grapheme-colour synesthesia with the question "Do numbers or letters cause you to have a colour experience?" A checkbox is provided for participants to record separately whether these colours are triggered automatically by numbers and/or digits. If participants indicated that they saw neither letters nor numbers in colour, they advanced to an exit page thanking them for their participation.

The consistency test was completed by participants who answered in the affirmative to having coloured letters/digits. This test displays individually on-screen the letters A-Z and/or the digits 0–9 (according to how participants responded to the checkboxes described above). Each grapheme is shown three times in a random order, and on each display, participants must indicate their synaesthetic colour by selecting it from an on-screen palette of 256x256x256 colours. The program compares the colour selected each time the same grapheme was presented (e.g., it compares the three colours for the letter A). It then produces a standardised score to reflect how far away in colour space those three colours were, averaged across all graphemes. A small standardised score reflects consistent colours (i.e., selections for the same grapheme were close in colour-space). A score less than 1 indicates the high level of consistency typical of

a synaesthete and this is the diagnostic threshold in this test. For full details regarding how this test is designed and implemented, please refer to Eagleman et al. (2007).

Results

In our study, we classified as non-synaesthetes all those who were directed to the early-exit page (i.e., those who said they did not experience coloured letters and/or digits) and all those who continued but scored 1 or higher. The remainder were classified as synaesthetes (i.e., those who scored <1).

From 3893 participants, we identified 54 grapheme-colour synaesthetes with coloured letters and/or digits (n = 5 with coloured letters; n = 26 with coloured digits; n = 23 with both coloured letters and digits), giving an overall prevalence of 1.39%. Of these 54 synaesthetes, 33 were female and 21 were male.[3] Calculating the overall prevalence of grapheme-colour synaesthesia for each sex separately taking into account the total number of men and women tested (2135 and 1758 respectively) gives us a female prevalence of 1.55% and a male prevalence of 1.19%, producing a female: male ratio of 1.3:1. This difference in the ratio of female versus male synaesthetes is not significant ($\chi^2 = 0.63$, $df = 1$, $p = 0.43$).[4]

Bayesian analysis

To further investigate our null result, we performed two types of Bayesian analyses below. Together these suggest that our sufficiently powered investigation of whether there is a 6:1 ratio gave strong support for the null hypothesis. However, they also provide an estimate of how small any possible female bias might yet be.

First, our Bayes factors analysis allows us to evaluate to what extent the data supports the hypothesis under investigation against the null hypothesis (Rouder, Speckman, Sun, Morey & Iverson, 2009). Following Jeffreys (1961), a Bayes factor of less than 0.33 provides strong support for the null hypothesis, a Bayes factor >3 provides support for the alternative hypothesis and values in between indicate no firm conclusions should be drawn. Our Bayes factor was 0.014, indicating strong support for the null hypothesis that sex does not significantly influence the prevalence of grapheme-colour synaesthesia.

Exploring our data further, a second analysis suggests that although there was no large significant difference across the sexes, there may yet be small difference, and we can calculate its size. We constructed a beta-binomial model of our acquired data which shows that any difference between the numbers of male and female synaesthetes in the general population is likely to be very small. Calculating a 95% confidence interval of the difference in prevalence, we see any difference in prevalence between females and males is likely to fall in the range −0.4% to 1.1%. Theoretically speaking, therefore, if we were confident that—say—our male prevalence of 1.19% were correct, we would therefore be 95% sure that the true female prevalence is in the small range between 0.79% (1.19%–0.4%) to 2.29% (1.19% +1.1%). Indeed, if there were a difference between men and women, our beta-binomial model also shows there is an 82% chance that the prevalence would be higher for females—albeit to this very marginal degree.

Discussion

We investigated the prevalence of grapheme-colour synaesthesia in males and females to challenge the suggestion that there are six times more female synaesthetes than male in the general population (Barnett et al., 2008; Baron-Cohen et al., 1996; Rich et al., 2005). First we pointed out that two previous studies showing this level of strong bias reported that their methodology relied on self-referral (e.g., Baron-Cohen et al., 1996; Rich et al., 2005). This method likely encouraged female synaesthetes to reply more than males (Simner et al., 2006; following Dindia & Allen, 1992). Second, we described how previous studies *not* liable to this confound (e.g., Simner et al., 2009, 2006) had *not* found a strong 6:1 bias towards females, and indeed had found no significant difference across the sexes at all. Third we examined an additional study showing a 6:1 bias of females which claimed not to rely on self-referral (Barnett et al., 2008). Using their published data and descriptions of study, we suggested that they may not have taken into account the total number of males/females tested overall or may not have used objective tests to verify synaesthesia in all

[3]Of the 33 female synaesthetes, 12 reported both coloured letters and digits, 5 reported experiencing coloured letters only and 16 reported coloured digits only. Of the 21 male, 11 reported both coloured letters and digits, 0 reported coloured letters only and 10 reported coloured digits only.

[4]If we examine the sex ratio for only synaesthetes who have both coloured letters AND numbers (n = 23; 12 female) we find a prevalence of 0.59%. Calculating the synaesthesia prevalence of each sex separately gives us a female prevalence of 0.56% and a male prevalence of 0.63%, and a ratio of 0.89: 1. Using a chi-squared test, we again determined the difference in ratio of female versus male synaesthetes is not significant ($\chi 2 = 0.002$, df = 1, p = 0.962).

TABLE 1

Shows the number of confirmed male (M) and female (F) grapheme-color synaesthetes found in our total sample of 3893 subjects (F = 2135; M = 1758). The prevalences are shown in brackets, with the female: male ratio beneath. This is done twice according to two different cut-off for synaesthesia (a score of 1 vs. 1.43 in *The Synesthesia Battery*) and twice according to two different definitions of grapheme-color synaesthesia (having colored letters AND/OR digits, vs. colored letters AND digits).

Coloured triggers	*Sex & ratio*	*Battery cut-off at 1*	*Battery cut-off at 1.43*
Letters AND/OR digits	F	33 (1.55%)	55 (2.58%)
	M	21 (1.19%)	39 (2.22%
	F + M	=54 (1.39%)	=94 (2.42%)
	Ratio F:M	**1.3:1**	**1.2:1**
Letters AND digits	F	12 (0.56%)	23 (1.08%)
	M	11 (0.63%)	19 (1.08%)
	F + M	=23 (0.59%)	=42 (1.08%)
	Ratio F:M	**0.89: 1**	**1:1**

participants, and that their methods did not appear to be entirely free of the self-referral confound.

In our empirical investigation, we screened 3893 individuals for grapheme-colour synaesthesia following a power analysis. We took care to avoid self-referral confounds and we individually tested every member of a pre-determined cohort with an objective test for synaesthesia. We found that 33 out of the 2135 females tested had grapheme-colour synaesthesia (for coloured letters and/or digits; female prevalence 1.55%) as well as 21 out of 1758 males (male prevalence 1.19%). This ratio of 1.3: 1, female to male synaesthetes, was non-significant. Further Bayes analyses suggest support for the null result in our data, but that there remains the possibility of a very small sex differences, in the range of −0.4% to 1.1%, with a female bias being more likely than a male bias.

Our results largely corroborate the findings of our previous comparison study, Simner et al. (2006) which reported an overall prevalence of grapheme-colour synaesthesia of 2%, compared to 1.39% in our own study (and this difference is non-significant; $\chi 2 = 1.16$, $p = .28$). This was for synaesthetes with coloured letters and/or digits, but it is also possible to directly compare our findings in the female: male ratio if we consider synaesthetes with both coloured letters *and* digits (since this is the type of sex data reported in Simner et al., 2006). In this comparison we find a female: male ratio of 0.9:1 in the current study compared to an identical ratio (0.9:1) found in Simner et al. (2006; their female prevalence was 1.03% and their male prevalence was 1.15%).

In our calculations we point out that we classified participants as synaesthetes according to the conventional cut-off, as stated within the test we used by Eagleman et al. (2007). This conventional cut-off for synaesthesia is a score <1. Two recent studies however have suggested that a more accurate approach might be a cut-off centred on 1.43 rather than 1 (for details see Carmichael et al., 2015; Rothen, Seth, Witzel & Ward, 2013). For this reason, we also re-calculate our prevalence and female/male ratio according to the 1.43 cut-off and find a yet-closer female: male ratio in synaesthesia. For clarity to aid the reader, we have presented this data along with our other prevalence/ratios in Table 1.

Of course we point out that our findings relate only to the sex ratio and prevalence of the population we sampled, and the type of synaesthesia we investigated. We note that our average sampled participant was 28 years old, which is younger than the national average (median = 40.5 years; Central Intelligence Agency, 2014), and this might have influenced the prevalence we generated. Furthermore, we looked only at grapheme-colour synaesthesia, which is just one of many variants of the condition (see Cytowic & Eagleman, 2009; Day, 2005). A recent study of a very large number of self-referred synaesthetes by Novich, Cheng and Eagleman (2011) revealed that groups of variants clusters into synaesthetic subtypes (e.g., people with grapheme-colour synaesthesia are likely to have a second form involving colour, but not taste). This suggests there may be multiple forms of the condition, and indicates in turn that what is true of grapheme-colour synaesthesia (e.g., its sex ratio) may not be representative of all synaesthesias.

One curiosity not yet understood is the apparent extent of the female bias in self-referral studies for synaesthesia. We have shown there are roughly equivalent numbers of female to male grapheme-colour synaesthesia in the general population—or at the very most, that there are only 1.3 women for every man. However, six times more female synaesthetes are detected in self-referral studies (e.g., Baron-Cohen et al. 1996). We attribute this difference in part to the known confound that promotes responses from women over men in self-referral (e.g., Dindia & Allen, 1992;

Rosenthal & Rosnow, 1975). However, Barnett et al. (2008) point out that this bias usually gives just a slight variation of around 10% (Dindia & Allen, 1992). Why then might the female bias be so exaggerated in studies of synaesthesia—and indeed, why are the rates so consistent across self-referral studies? It could be, for example, that female synaesthetes—although not greatly more common—have perhaps more intense experiences or are more aware of their synaesthesia or attend to it more in daily life. This might make them more likely to self-refer. However, we have no data to support any specific supposition in this area, so leave this question for future investigations.

Understanding sex ratios are important in understanding the origins of synaesthesia. Initial findings that synaesthesia appeared more common in females led to suggestions that synaesthesia was either not fully expressed in males or that it was linked to the X-chromosome in some way (Bailey & Johnson, 1997; Baron-Cohen et al., 1996; Ward & Simner, 2005). Indeed, the extent of the female bias led researchers to propose that synaesthesia might causes lethality in males *in utero* (Baron-Cohen et al., 1996). Subsequent research, including our own study here, suggests this is not the case. In combination with previous studies from our own lab and elsewhere, we conclude there no very strong female bias (Simner et al., 2009, 2006), that families containing synaesthetes are equally likely to produce female or male offspring (Barnett et al., 2008; Ward & Simner, 2005), that there are confirmed cases of male-to-male transmission (Asher et al., 2009), and one case of monozygotic male twins who are discordant for synaesthesia (Smilek, Dixon & Merikle, 2005). Finally, neither Asher et al. (2009) nor Tomson et al. (2011) found evidence for a major locus on the X chromosome in their genome-wide studies.[5] This suggests a need to revisit our early understanding of the mode of inheritance of synesthesia (see Asher & Carmichael, 2013, for review) and we provide our data for future studies to do so.

We finally point out that our own studies have shown relatively flat distributions of synaesthesia in men and women, with a slight male bias when considering grapheme-colour synaesthetes with coloured letters *and* digits (female: male ratio of 0.9: 1 both here and in Simner et al., 2006) and a slight female bias when considering grapheme-colour synaesthetes with coloured letters *and/or* digits (here, female: male ratio of 1.3: 1). It may yet be possible to estimate the numbers required to test this much reduced difference across the sexes (e.g., power analyses in the ratio of 1.3:1 suggest we would need to screen 47516 participants) but for the current study we have shown that there is no 6:1 ratio of female to male synaesthetes, even with sufficient power to test for such a difference.

[5]Both these genetics studies screened probands and family members for synaesthesia but no information about the sex of participants was given in Asher et al. (2009). Although Tomson et al. (2013) did report the sex of their participants, they screened just 5 families giving too few datapoints to draw firm conclusions from their female: male ratio (2.7:1) which was, furthermore, generated via a self-referral bias in probands. These genetics studies therefore serve an important purpose in describing the genetic aetiology of synaesthesia but cannot be used to empirically authenticate sex-ratios.

Original manuscript received 1 December 2014
Revised manuscript received 12 January 2015
First published online 13 March 2015

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